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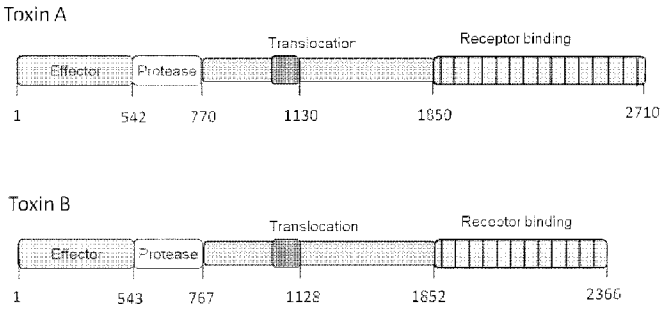

(57) Abrégé/Abstract:
The present invention relates to recombinant Clostridium difficile antigens based on a fusion protein that consists of or comprises a first amino acid sequence and a second amino acid sequence, wherein: a) the first amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 500-1850 of a C. difficile Toxin A sequence or residues 1500-1851 of a C. difficile Toxin B sequence; and b) the second amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1851-2710 of a C. difficile Toxin A sequence or within amino acid residues 1852-2366 of a C. difficile Toxin B sequence; though with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2710 of a C. difficile Toxin A and with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a C. difficile Toxin B. Also provided is the use of said antigens for the prevention/ treatment/ suppression of Clostridium difficile infection (CDI), together with methods for generating said antigens, methods for generating antibodies that bind to said antigens, and the use of said antibodies for the prevention/ treatment/ suppression of CDI.

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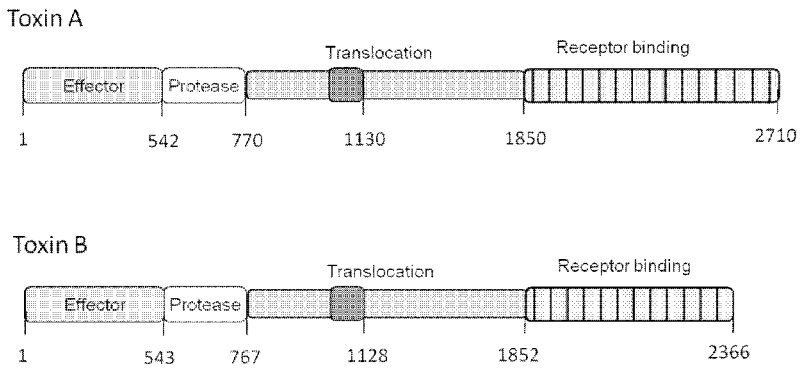
(54) **Title**: CLOSTRIDIUM DIFFICILE ANTIGENS

Figure 1



(57) **Abstract**: The present invention relates to recombinant *Clostridium difficile* antigens based on a fusion protein that consists of or comprises a first amino acid sequence and a second amino acid sequence, wherein: a) the first amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 500-1850 of a *C. difficile Toxin* A sequence or residues 1500-1851 of a *C. difficile* Toxin B sequence; and b) the second amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1851-2710 of a *C. difficile Toxin* A sequence or within amino acid residues 1852-2366 of a *C. difficile Toxin* B sequence; though with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2710 of a *C. difficile Toxin* A and with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a *C. difficile Toxin* B. Also provided is the use of said antigens for the prevention/ treatment/ suppression of Clostridium difficile infection (CDI), together with methods for generating said antigens, methods for generating antibodies that bind to said antigens, and the use of said antibodies for the prevention/ treatment/ suppression of CDI.

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Clostridium difficile antigens

The present invention relates to antigens for the prevention/ treatment/ suppression of *Clostridium difficile* infection (CDI). Also provided are methods for generating said antigens, methods for generating antibodies that bind to said antigens, and the use of said antibodies for the prevention/ treatment/ suppression of CDI.

Clostridium difficile infection (CDI) is now a major problem in hospitals worldwide. The bacterium causes nosocomial, antibiotic-associated disease which manifests itself in several forms ranging from mild self-limiting diarrhoea to potentially life-threatening, severe colitis. Elderly patients are most at risk from these potentially life-threatening diseases and incidents of CDI have increased dramatically over the last 10 years. In 2010 in the UK there were over 21,000 cases of CDI with over 2,700 associated deaths. CDI costs the UK National Health Service in excess of £500M per annum.

The various strains of *C. difficile* may be classified by a number of methods. One of the most commonly used is polymerase chain reaction (PCR) ribotyping in which PCR is used to amplify the 16S-23S rRNA gene intergenic spacer region of *C. difficile*. Reaction products from this provide characteristic band patterns identifying the bacterial ribotype of isolates. Toxinotyping is another typing method in which the restriction patterns derived from DNA coding for the *C. difficile* toxins are used to identify strain toxinotype. The differences in restriction patterns observed between toxin genes of different strains are also indicative of sequence variation within the *C. difficile* toxin family. For example, there is an approximate 13% sequence difference with the C-terminal 60kDa region of toxinotype 0 Toxin B compared to the same region in toxinotype III Toxin B.

Strains of *C. difficile* produce a variety of virulence factors, notable among which are several protein toxins: Toxin A, Toxin B and, in some strains, a binary toxin which is similar to *Clostridium perfringens* iota toxin. Toxin A is a large protein cytotoxin/ enterotoxin which plays a role in the pathology of infection and may influence the gut colonisation process. Outbreaks of CDI have been reported with Toxin A-negative/Toxin B-positive strains, which indicates that Toxin B is also capable of playing a key role in the disease pathology.

The genetic sequences encoding Toxin A and Toxin B (Mw 308k and Mw 269k, respectively) are known - see, for example, Moncrief *et al.* (1997) Infect. Immun 63: 1105-1108. The two toxins have high sequence homology and are believed to have arisen from gene duplication. The toxins also share a common structure (see Figure 1), namely an N-terminal glucosyl

transferase domain, a central hydrophobic region, four conserved cysteines, and a long series of C-terminal repeating units (RUs).

Toxin A comprises 39 contiguous repeating units (RUs), which span amino acid residues 1851-2710 of the Toxin A polypeptide sequence. Toxin B comprises fewer RUs (between 19 and 24) which span amino acid residues 1852-2366 of the Toxin B polypeptide sequence. For both Toxins A and B, the repeating units are of two different types: short repeats (SRs) of approximately 15-25 residues and long repeats (LRs) of approximately 30 residues. The LRs are separated from each other by 3 or 4 SRs, and the LRs together with the flanking SRs provide the binding sites for the carbohydrate receptor of the toxins. Toxin A has 7 LRs within its C-terminal domain, which are believed to provide 7 receptor binding sites (Greco et al. (2005) Nature Structural Biol. 13: 460-461). Toxin B has 4 LRs, which are believed to provide 4 carbohydrate binding units. Examples of the Toxin A and Toxin B SR/LR clusters (also known as receptor-binding "Modules") vary in size from 92-141 amino acid residues, and are exemplified by reference to Tables 1 and 2.

Both Toxins A and B exert their mechanisms of action via multi-step mechanisms, which include binding to receptors on the cell surface, internalisation followed by translocation and release of the effector domain into the cell cytosol, and finally intracellular action. Said mechanism of action involves the inactivation of small GTPases of the Rho family. In this regard, the toxins catalyse the transfer of a glucose moiety (from UDP-glucose) onto an amino residue of the Rho protein. Toxins A and B also contain a second enzyme activity in the form of a cysteine protease, which appears to play a role in the release of the effector domain into the cytosol after translocation. The *C. difficile* binary toxin modifies cell actin by a mechanism which involves the transfer of an ADP-ribose moiety from NAD onto its target protein.

Current therapies for the treatment of *C. difficile* infection rely on the use of antibiotics, notably metronidazole and vancomycin. However, these antibiotics are not effective in all cases and 20-30% of patients suffer relapse of the disease. Of major concern is the appearance in the UK of more virulent strains, which were first identified in Canada in 2002. These strains, which include those belonging to PCR ribotype 027 and toxinotype III, cause CDI with a directly attributable mortality more than 3-fold that observed previously.

New therapeutics are therefore required especially urgently since the efficacy of current antibiotics appears to be decreasing.

An attractive alternative is the use of antibodies which bind to and neutralise the activity of Toxin A and Toxin B. This is based on the knowledge that strains of *C. difficile* that do not release these toxins, so called non-toxigenic strains, do not cause CDI. In one approach patients with CDI or subjects at risk of developing such infections can be immunised with antigens which result in an increase in circulating and mucosal antibodies directed against Toxin A and Toxin B. This is defined as active immunisation. Alternatively, animals, such as horses or sheep, can be immunised, their sera collected and the antibodies purified for administration to patients - passive immunisation.

A critical requirement for both active and passive immunisation is the availability of suitable antigens with which to immunise the patient or animal respectively. These can comprise the natural toxins which can be purified from the media in which suitable toxigenic strains of *C. difficile* have been cultured. There are several disadvantages to this approach. Both Toxin A and Toxin B are present in culture medium in only small amounts and are difficult to purify without incurring significant losses. Thus, it will be both costly and difficult to obtain the amounts necessary to meet world-wide needs. In addition, the natural toxins are unstable and, because of their toxicity, must be converted to their toxoids (inactivated toxins) prior to their use as immunogens.

The above mentioned problems have resulted in there being few available *C. difficile* vaccine candidates. To-date, the only CDI vaccine in late-stage development is based on a mixture of native (i.e. naturally occurring) Toxins A and B, which have been inactivated by chemical modification (Salnikova et al 2008, J Pharm Sci 97: 3735-3752)

One alternative to the use of natural toxins and their toxoids, involves the design, development and use of recombinant fragments derived from Toxins A and B. Among their advantages are that such fragments can be expressed and purified in large amounts and at lower cost than the native toxins. Examples of existing antigens intended for use in treating/ preventing a *C. difficile* infection include peptides based on the C-terminal repeating units (RUs) of Toxin A or Toxin B – see, for example, WO00/61762. A problem with such antigens, however, is that they are either poorly immunogenic (i.e. the antigens produce poor antibody titres), or, where higher antibody titres are produced, the antibodies demonstrate poor neutralising efficacy against *C. difficile* cytotoxic activity (i.e. insufficient neutralising antibodies are produced).

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There is therefore a need in the art for new vaccines/ therapies/ therapeutics capable of specifically addressing *C. difficile* infection (CDI). This need is addressed by the present invention, which solves one or more of the above-mentioned problems.

In one embodiment, the present invention provides antigens that are able to induce a potent toxin-neutralising response against C. *difficile* Toxin A and/ or B. The invention also provides methods for preparing recombinant antigens. In another embodiment, said antigens are used as immunogens to enable the large-scale preparation of therapeutic antibodies. In a further embodiment, said antibodies are able to induce a potent toxin-neutralising response against C. *difficile* Toxin A and/ or B and therefore have prophylactic and/ or therapeutic applications.

As mentioned above (see WO 00/61762), previous studies describe vaccine preparations based on the C-terminal, repeating units (RUs) of Toxin A and/ or Toxin B. Said RU fragments have a poor toxin-neutralising effect, and/ or are difficult to manufacture in large quantities.

In contrast, the present invention provides a C. *difficile* antigen based on a Toxin A and/ or a Toxin B repeat unit, and further includes an additional C. *difficile* toxin domain, which the present inventors believe provides an important 'scaffold' function to the antigen. Said antigens of the invention demonstrate good toxin-neutralising immune responses and/ or are readily manufactured in large quantities.

The present inventors have surprisingly identified that the presence of a "scaffold" first amino acid sequence (as above) provides a protective (toxin-neutralising) immune response that is between 10-100 fold increased as compared to corresponding fragments comprising just the repeat regions of Toxin A or Toxin B. Tables 3-10 clearly show the superior capacity of fusion proteins of the present invention to elicit a toxin-neutralising immune response compared to fragments containing just the repeat domains of a *C. difficile* Toxin. Comparison of the data in Tables 5 and 6 confirms that the Toxin B-based constructs of the present invention elicit a considerably more potent toxin-neutralising immune response than that of a corresponding construct based solely on the C-terminal repeating units of Toxin B (designated TxB2). In more detail, after an 18-week immunisation period, the toxin-neutralising immune response provided by constructs of the present invention was approximately 128-fold higher than that provided by the TxB2 construct. Tables 9 and 10 show similar data for Toxin A-based constructs of the present invention. Comparison of the data in said Tables confirms that the Toxin A-based constructs of the present invention elicit

a considerably more potent toxin-neutralising immune response than that of a corresponding construct based solely on the C-terminal repeating units of Toxin A (designated TxA2). In more detail, after an 18-week immunisation period, the toxin-neutralising immune response provided by constructs of the present invention was 12-fold higher than that provided by the TxA2 construct.

These findings are surprising for a number of reasons. Previous studies have shown that toxin fragments consisting of the *C. difficile* Toxin RUs fold correctly, readily crystallise to yield an ordered structure (Ho *et al.* (2005) PNAS, 102: 18373-18378), and bind carbohydrate moieties that mimic the natural *C. difficile* Toxin receptors (Greco *et al.* (2006) Nature Structure. & Molecular Biology, 13: 460-461). Thus, the scientific evidence to-date supports and is consistent with the prior art use (e.g. WO 00/61762) of fragments consisting of the *C. difficile* Toxin RUs in antigenic formulations. More importantly, however, a further study has confirmed that antibodies raised against a whole *C. difficile*, while recognising a fragment consisting of the entire RU region alone, failed to recognise a fragment consisting of a "scaffold" region based on residues 901-1750 of the *C. difficile* same toxin (Genth *et al.*, (2000) Infect. Immun., 68: 1094-1101). These data therefore suggest that domains within "scaffold" residues 901-1750 contribute no significant antibody-binding structural determinants. In this regard, other than at the peptide bond, there is no contact in the tertiary structure between "scaffold" toxin domains and the C-terminal repeat region residues – see Pruitt *et al.,* (2010) PNAS 1002199107 online publication. Collectively, it is therefore extremely surprising that the inclusion of a *C. difficile* "scaffold" region within recombinant immunogens of Toxins A and/ or Toxin B has the effect of significantly enhancing the toxin-neutralising immune response.

A first aspect of the present invention provides a fusion protein, consisting of or comprising a first amino acid sequence and a second amino acid sequence, wherein:

1) the first amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 1500-1850 of a *C. difficile* Toxin A sequence; and

2) the second amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1851-2710 of a *C. difficile* Toxin A sequence;

with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2710 of a *C. difficile* Toxin A.

Reference to a *C. difficile* Toxin A sequence means the amino acid sequence of a naturally-occurring *C. difficile* Toxin A (also referred to as a *C. difficile* Toxin A reference sequence). Examples of such sequences are readily understood by a skilled person, and some of the more common naturally-occurring Toxin A sequences are identified in the present specification (see, for example, SEQ ID NOs: 1 & 3) as well as throughout the literature.

Reference to 'at least 80% sequence identity' throughout this specification is considered synonymous with the phrase 'based on' and may embrace one or more of at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 99%, and 100% sequence identity. When assessing sequence identity, a reference sequence having a defined number of contiguous amino acid residues is aligned with an amino acid sequence (having the same number of contiguous amino acid residues) from the corresponding portion of a fusion protein of the present invention.

In one embodiment, the first amino acid sequence is based on (ie. has at least 80% sequence identity with) amino acid residues 544-1850 of a *C. difficile* Toxin A. In another embodiment, the first amino acid sequence is based on an N-terminal truncation of amino acid residues 544-1850 of a *C. difficile* Toxin A, such as amino acid residues 564-1850, amino acid residues 584-1850, amino acid residues 594-1850, amino acid residues 614-1850, amino acid residues 634-1850, amino acid residues 654-1850, amino acid residues 674-1850, amino acid residues 694-1850, amino acid residues 714-1850, amino acid residues 734-1850, amino acid residues 754-1850, amino acid residues 767-1850, amino acid residues 770-1850, amino acid residues 774-1850, amino acid residues 794-1850, amino acid residues 814-1850, amino acid residues 834-1850, amino acid residues 854-1850, amino acid residues 874-1850, amino acid residues 894-1850, amino acid residues 914-1850, amino acid residues 934-1850, amino acid residues 954-1850, amino acid residues 974-1850, amino acid residues 994-1850, amino acid residues 1014-1850, amino acid residues 1034-1850, amino acid residues 1054-1850, amino acid residues 1074-1850, amino acid residues 1094-1850, amino acid residues 1104-1850, amino acid residues 1124-1850, amino acid residues, amino acid residues 1131-1850, amino acid residues 1144-1850, amino acid residues 1164-1850, amino acid residues 1184-1850, amino acid residues 1204-1850, amino acid residues 1224-1850, amino acid residues 1244-1850, amino acid residues 1264-1850, amino acid residues 1284-1850, amino acid residues 1304-1850, amino acid residues 1324-1850, amino acid residues 1344-1850, amino acid residues 1364-1850, amino acid residues 1384-1850, amino acid residues 1404-1850, amino acid residues 1424-1850, amino acid residues 1444-1850, amino acid residues 1464-1850, or amino acid residues 1684-1850 of a *C. difficile* Toxin A; though always with the proviso that the fusion

protein is not a polypeptide comprising amino acid residues 543-2710 of a *C. difficile* Toxin A. By way of example only, the above amino acid position numbering may refer to the *C. difficile* Toxin A sequences identified as SEQ ID NOs: 1 and/ or 3.

In one embodiment, the second amino acid sequence is based on (ie. has at least 80% sequence identity with) any one or more of the long repeat (LR) amino acid sequences from a *C. difficile* Toxin A sequence. By way of example only, said one or more LR sequences may be based on any of SEQ ID NOs: 60, 62, 64, 66, 68, 70 and/ or 72. In another embodiment, the second amino acid sequence is based on an entire Module sequence of a *C. difficile* Toxin A sequence, which includes a LR amino acid sequence plus one or more of its (flanking) short repeat (SR) sequences. By way of example only, the second amino acid may be based on one or more of SEQ ID NOs: 61, 63, 65, 67, 69, 71 and/ or 73. In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 amino acid sequence from a *C. difficile* Toxin A sequence (residues 1851-2007) – see, for example, the Module 1 as illustrated in Table 1. In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 amino acid sequence from a *C. difficile* Toxin A sequence (eg. residues 1851-2141 as illustrated in Table 1). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 amino acid sequence from a *C. difficile* Toxin A sequence (eg. residues 1851-2253 as illustrated in Table 1). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 plus Module 4 amino acid sequence from a *C. difficile* Toxin A sequence (eg. residues 1851-2389 as illustrated in Table 1). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 plus Module 4 plus Module 5 amino acid sequence from a *C. difficile* Toxin A sequence (eg. residues 1851-2502 as illustrated in Table 1). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 plus Module 4 plus Module 5 plus Module 6 amino acid sequence from a *C. difficile* Toxin A sequence (eg. residues 1851-2594 as illustrated in Table 1). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 plus Module 4 plus Module 5 plus Module 6 plus Module 7 amino acid sequence from a *C. difficile* Toxin A sequence (eg. residues 1851-2710 as illustrated in Table 1). By way of example only, the above amino acid position numbering may refer to the *C. difficile* Toxin A sequences identified as SEQ ID NOs: 1 and/ or 3.

Any of the embodiments for the second amino acid sequence may be combined with any of the embodiments described for the first amino acid sequence.

In one embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1851-2710 of a Toxin A sequence (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 770-1850 of a Toxin A polypeptide (or a portion thereof).

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1851-2710 of a Toxin A sequence (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 1131-1850 of a Toxin A polypeptide.

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 770-2710 or 1131-2710 of a Toxin A polypeptide (e.g. SEQ ID NOs 5, 6, 7, 8, 18, 19, 20, 21, 22, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, or 58).

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 770-2007, 770-2141, 770-2253, 770-2389 or 1131-2007, 1131-2141, 1131-2253 or 1131-2389 of a Toxin A polypeptide (e.g. SEQ ID NO 59).

A related first aspect of the present invention provides a fusion protein, consisting of or comprising a first amino acid sequence and a second amino acid sequence, wherein:
1) the first amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 1500-1851 of a *C. difficile* Toxin B sequence; and
2) the second amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1852-2366 of a *C. difficile* Toxin B sequence;
with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a *C. difficile* Toxin B.

Reference to a *C. difficile* Toxin B sequence means the amino acid sequence of a naturally-occurring *C. difficile* Toxin B (also referred to as a *C. difficile* Toxin B reference sequence). Examples of such sequences are readily understood by a skilled person, and some of the

more common naturally-occurring Toxin B sequences are identified in the present specification (see, for example, SEQ ID NOs: 2 & 4) as well as throughout the literature.

In one embodiment, the first amino acid sequence is based on (ie. has at least 80% sequence identity with) amino acid residues 544-1851 of a *C. difficile* Toxin B. In another embodiment, the first amino acid sequence is based on an N-terminal truncation of amino acid residues 544-1851 of a *C. difficile* Toxin B, such as amino acid residues 564-1851, amino acid residues 584-1851, amino acid residues 594-1851, amino acid residues 614-1851, amino acid residues 634-1851, amino acid residues 654-1851, amino acid residues 674-1851, amino acid residues 694-1851, amino acid residues 714-1851, amino acid residues 734-1851, amino acid residues 754-1851, amino acid residues 767-1851, amino acid residues 770-1851, amino acid residues 774-1851, amino acid residues 794-1851, amino acid residues 814-1851, amino acid residues 834-1851, amino acid residues 854-1851, amino acid residues 874-1851, amino acid residues 894-1851, amino acid residues 914-1851, amino acid residues 934-1851, amino acid residues 954-1851, amino acid residues 974-1851, amino acid residues 994-1851, amino acid residues 1014-1851, amino acid residues 1034-1851, amino acid residues 1054-1851, amino acid residues 1074-1851, amino acid residues 1094-1851, amino acid residues 1104-1851, amino acid residues 1124-1851, amino acid residues 1131-1851, amino acid residues 1144-1851, amino acid residues 1164-1851, amino acid residues 1184-1851, amino acid residues 1204-1851, amino acid residues 1224-1851, amino acid residues 1244-1851, amino acid residues 1264-1851, amino acid residues 1284-1851, amino acid residues 1304-1851, amino acid residues 1324-1851, amino acid residues 1344-1851, amino acid residues 1364-1851, amino acid residues 1384-1851, amino acid residues 1404-1851, amino acid residues 1424-1851, amino acid residues 1444-1851, amino acid residues 1464-1851, or amino acid residues 1684-1851 of a *C. difficile* Toxin B; though always with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a *C. difficile* Toxin B. By way of example only, the above amino acid position numbering may refer to the *C. difficile* Toxin B sequences identified as SEQ ID NOs: 2 and/ or 4.

In one embodiment, the second amino acid sequence is based on (ie. has at least 80% sequence identity with) any one or more of the long repeat (LR) amino acid sequences from a *C. difficile* Toxin B sequence. By way of example only, said one or more LR sequences may be based on any of SEQ ID NOs: 74, 76, 78 and/ or 80. In another embodiment, the second amino acid sequence is based on an entire Module sequence of a *C. difficile* Toxin B sequence, which includes a LR amino acid sequence plus one or more of its (flanking) short repeat (SR) sequences. By way of example only, the second amino acid sequence may be

based on one or more of SEQ ID NOs: 75, 77, 79 and/ or 81. In another embodiment the second amino acid is based on a sequence consisting of or comprising the entire Module 1 amino acid sequence from a *C. difficile* Toxin B sequence (residues 1852-2007) – see, for example, the Module 1 as illustrated in Table 2. In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 amino acid sequence from a *C. difficile* Toxin B sequence (eg. residues 1852-2139 as illustrated in Table 2). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 amino acid sequence from a *C. difficile* Toxin B sequence (eg. residues 1851-2273 as illustrated in Table 2). In another embodiment, the second amino acid sequence is based on a sequence consisting of or comprising the entire Module 1 plus Module 2 plus Module 3 plus Module 4 amino acid sequence from a *C. difficile* Toxin B sequence (eg. residues 1851-2366 as illustrated in Table 2). By way of example only, the above amino acid position numbering may refer to the *C. difficile* Toxin B sequences identified as SEQ ID NOs: 2 and/ or 4.

Any of the embodiments for the second amino acid sequence may be combined with any of the embodiments described for the first amino acid sequence.

In one embodiment, when the first and second amino acid sequences are both based on Toxin B sequences, the fusion protein may consist of or comprise an amino acid sequence that is based on at least 871 or at least 876 or at least 881 or at least 886 or at least 891 or at least 896 or at least 901 contiguous amino acid residues (e.g. starting from the C-terminal amino acid residue) of a *C. difficile* Toxin B sequence, such as SEQ ID NOs: 2 and/ or 4).

In one embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1852-2366 of a Toxin B polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 767-1851 of a Toxin B polypeptide (or a portion thereof).

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1852-2366 of a Toxin B polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 1145-1851 of a Toxin B polypeptide (or a portion thereof).

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 767-2366 or 957-2366 or 1138-2366 of a Toxin B

polypeptide (e.g. SEQ ID NOs 9, 10, 11, 12, 13, 14, 23, 24, 25, 26, 27, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 57).

The present invention also provides fusion proteins that are chimeras of Toxin A and B domains. For example, one or more long repeat unit (optionally including one or more short repeat unit; or one, more or all Modules) based on a Toxin B polypeptide may be combined with a "scaffold" region of a Toxin A polypeptide. Similarly, one or more long repeat unit (optionally including one or more short repeat unit; or one, more or all Modules) based on a Toxin A polypeptide may be combined with a "scaffold" region of a Toxin B polypeptide.

Thus, a further related aspect of the present invention provides a hybrid/ chimera fusion protein, consisting of or comprising a first amino acid sequence and a second amino acid sequence, wherein:
1) the first amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 1500-1850 of a *C. difficile* Toxin A sequence; and
2) the second amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1852-2366 of a *C. difficile* Toxin B sequence;
with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2710 of a *C. difficile* Toxin A;
and with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a *C. difficile* Toxin B.

Embodiments of the first and second amino acid sequences are as detailed above.

For example, in one embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1852-2366 of a Toxin B polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 770-1849 of a Toxin A polypeptide (or a portion thereof).

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1852-2366 of a Toxin B polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 1131-1849 of a Toxin A polypeptide (or a portion thereof).

In another embodiment, a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1852-2366 of a Toxin B polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 1500-1849 of a Toxin A polypeptide (or a portion thereof). In one embodiment, said Toxin A polypeptide component is preferably based on a sequence that is shorter than residues 543-1849 of a Toxin A polypeptide.

Specific examples include fusion proteins consisting of or comprising an amino acid sequence based on any one or more of SEQ ID NOs: 16 or 17.

Similarly, a further related first aspect of the present invention provides a hybrid/ chimera fusion protein, consisting of or comprising a first amino acid sequence and a second amino acid sequence, wherein:
1) the first amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 1500-1851 of a *C. difficile* Toxin B sequence; and
2) the second amino acid sequence is provided by an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1851-2710 of a *C. difficile* Toxin A sequence;
with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2710 of a *C. difficile* Toxin A
and with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a *C. difficile* Toxin B.

Embodiments of the first and second amino acid sequences are as detailed above.

In one embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1850-2710 of a Toxin A polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 767-1851 of a Toxin B polypeptide (or a portion thereof).

In another embodiment a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1850-2710 of a Toxin A polypeptide (or a portion thereof) and an N-terminal polypeptide based on amino acid residues 1145-1851 of a Toxin B polypeptide (or a portion thereof).

In another embodiment, a fusion protein is provided, which comprises or consists of a sequence based on amino acid residues 1850-2710 of a Toxin A polypeptide (or a portion thereof) and an N-terminal polypeptide based on 1500-1851 of a Toxin B polypeptide. In one embodiment, the Toxin B polypeptide component is preferably based on a sequence that is shorter than residues 543-1851 of a Toxin B polypeptide.

Specific examples include fusion proteins consisting of or comprising an amino acid sequence based on SEQ ID NO: 15.

As hereinbefore described, the present invention relates to fusion proteins based on a "scaffold" section plus a LR portion (of the C-terminal repeating units) of a *C. difficile* Toxin A and/ or a *C. difficile* Toxin B. In this regard, the total portion(s) of said fusion proteins that is based on said *C. difficile* Toxin A and/ or Toxin B sequences typically amounts to a maximum of 1940 contiguous amino acid residues (for example a maximum of 1890, or 1840, or 1790, or 1740, or 1690, or 1640, or 1590, or 1540, or 1490, 1440, or 1390, or 1340, or 1290, or 1240 contiguous amino acid residues).

In one embodiment, the fusion protein substantially lacks cysteine protease activity. In another (or the same) embodiment, the fusion protein substantially lacks glucosyl transferase activity. For example, part or all of the amino acid sequence(s) providing said activity (activities) are typically absent (e.g. deleted) from the fusion proteins of the present invention. These enzymatic activities are present in native Toxin A and/ or Toxin B, and are associated with N-terminal domains of said Toxins (see Figure 1).

In another embodiment, the fusion protein substantially lacks the glucosyl transferase domain (amino acid residues 1-542 Toxin A; amino acid residues 1-543 Toxin B) of a native *C. difficile* Toxin. In another (or the same) embodiment, the fusion protein substantially lacks the cysteine protease domain (amino acid residues 543-770 Toxin A; 544-767 Toxin B) of a native *C. difficile* Toxin. Said amino acid residue numbering refers to any Toxin A or Toxin B toxinotype, for example any one or more of the reference Toxin A and/ or Toxin B toxinotype SEQ ID NOs recited in the present specification. Accordingly, said amino acid residue numbering may refer to any specific Toxin A and/ or Toxin B reference SEQ ID NO recited in the present specification including an amino acid sequence variant having at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, or at least 99% thereto.

Fusion protein constructs of the invention may be derived from any Toxin A and/ or B sequence (including any toxinotype sequence), such as those illustrated in the present

specification. For example, in one embodiment, first and/ or second amino acid sequences are derived from Toxins A and/ or B of toxinotype 0 (SEQ IDs 1 and 2, respectively). In another embodiment, first and/ or second amino acid sequences are derived from Toxins A and/ or B of toxinotype 3 (SEQ IDs 3 and 4, respectively).

Fusion proteins of the invention may further comprise a fusion protein partner to facilitate soluble expression. Fusion protein partners may be attached at the N- or C-terminus of the antigen construct but are usually placed at the N-terminal end. Examples of fusion partners are: NusA, thioredoxin, maltose-binding protein, small ubiquitin-like molecules (Sumo-tag). To facilitate removal of the fusion protein partner during purification, a unique protease site may be inserted between the fusion protein partner and the fusion protein *per se*. Such protease sites may include those for thrombin, factor Xa, enterokinase, PreScission™, Sumo™. Alternatively, removal of the fusion protein partner may be achieved via inclusion of an intein sequence between the fusion protein partner and the fusion protein *per se*. Inteins are self cleaving proteins and in response to a stimulus (e.g. lowered pH) are capable of self splicing at the junction between the intein and the antigen construct thus eliminating the need for the addition of specific proteases. Examples of inteins include domains derived from *Mycobacterium tuberculosis* (RecA), and *Pyrococcus horikoshii* (RadA) (Fong et al. (2010) Trends Biotechnol. 28:272-279).

To facilitate purification, fusion proteins of the invention may include one or more purification tags to enable specific chromatography steps (e.g. metal ion chelating, affinity chromatography) to be included in the purification processes. Such purification tags may, for example, include: repeat histidine residues (e.g. 6-10 histidine residues), maltose binding protein, glutathione S-transferase; and streptavidin. These tags may be attached at the N- and/ or C-terminus of the antigen fusion proteins of the invention. To facilitate removal of such tags during purification, protease sites and/ or inteins (examples above) may be inserted between the fusion protein and the purification tag(s).

Thus, a typical fusion protein construct of the invention (starting from the N-terminus) may comprise:

- a first purification tag
- a fusion protein partner (to facilitate expression)
- a first (preferably specific) protease sequence or intein sequence
- the Toxin A and/ or B antigen sequence
- an optional second (preferably specific) protease sequence or intein sequence
- an optional second purification tag

The first and second purification tags may be the same or different. Similarly, the first and second protease/ intein sequence may be the same or different. The first and second options are preferably different to enable selective and controllable cleavage/ purification.

Specific examples of such fusion protein constructs are show in SEQ IDs 18-27.

In one embodiment spacers may be introduced to distance the purification tag from the fusion protein – this may help to increase binding efficiency to affinity purification column media. The spacer may be placed (immediately) after the purification tag or between the fusion protein partner and the fusion protein *per se*. Typical spacer sequences may consist of between 10-40 amino acid residues to give either a linear or alpha-helical structure.

Accordingly, in one embodiment, a fusion protein construct of the invention may comprise (starting from the N-terminus):
- a first purification tag
- an optional first spacer sequence
- a fusion protein partner (to facilitate expression)
- an optional second spacer sequence
- a (preferably specific) protease sequence or intein sequence
- the Toxin A and/ or B derived antigen sequence
- an optional second (preferably specific) protease sequence or intein sequence
- an optional third spacer sequence
- an optional second purification tag

Specific examples of such protein fusion constructs are show in SEQ IDs 28-57

Genes encoding the constructs of the invention may be generated by PCR from *C. difficile* genomic DNA and sequenced by standard methods to ensure integrity. Alternatively and preferably genes may be synthesised providing the optimal codon bias for the expression host (e.g. *E. coli*, *Bacillus megaterium*). Thus, the present invention provides corresponding nucleic acid sequences that encode the aforementioned fusion proteins of the present invention.

Accordingly, a second aspect of the present invention provides a method for expressing one or more of the aforementioned fusion proteins, said method comprising:

1) providing a nucleic acid sequence that encodes one or more of said fusion proteins in a host cell, wherein said nucleic acid sequence is operably linked to a promoter; and
2) expressing said nucleic acid sequence in the host cell

Fusion proteins of the invention may be formulated as vaccines for human or animal use in a number of ways. For example, formulation may include treatment with an agent to introduce intra-molecular cross-links. One example of such an agent is formaldehyde, which may be incubated, for example, with antigen fusion proteins of the invention for between 1-24 hours. Alternatively, longer incubation times of, for example, up to 2, 4, 6, 8 or 10 days may be employed. Following treatment with such an agent, antigen fusions of the invention may be combined with a suitable adjuvant, which may differ depending on whether the antigen fusion protein is intended for human or animal use.

A human or animal vaccine formulation may contain Toxin A and/ or Toxin B and/ or corresponding hybrid/ chimera antigen fusions of the present invention. Thus, in one embodiment, a vaccine formulation procedure of the present invention comprises the following steps:

- providing a recombinant Toxin A and/ or Toxin B and/ or hybrid/ chimera toxin fusion protein in suitable buffer system
- optionally (preferably) treating said mixture with a toxoiding component such as formaldehyde
- optionally transferring the fusion proteins to a new buffer system
- combining the fusion proteins with one or more suitable adjuvants and optionally other excipients

Accordingly, a third aspect of the present invention provides one or more of the aforementioned fusion proteins of the invention, for use in the generation of antibodies that bind to *C. difficile* Toxin A and/ or Toxin B. In one embodiment, said antibodies bind to and neutralise *C. difficile* Toxin A and/ or Toxin B.

For immunisation of animals, the *C. difficile* recombinant fusion protein antigens of the invention may be used as immunogens separately or in combination, either concurrently or sequentially, in order to produce antibodies specific for individual *C. difficile* toxins or combinations. For example, two or more recombinant antigens may be mixed together and used as a single immunogen. Alternatively a *C. difficile* toxin fusion protein antigen (e.g. Toxin A-derived) may be used separately as a first immunogen on a first animal group, and another *C. difficile* toxin antigen (e.g. Toxin B-derived) may be used separately on a second

animal group. The antibodies produced by separate immunisation may be combined to yield an antibody composition directed against *C. difficile* toxins. Non-limiting examples of suitable adjuvants for animal/veterinary use include Freund's (complete and incomplete forms), alum (aluminium phosphate or aluminium hydroxide), saponin and its purified component Quil A.

A fourth (vaccine) aspect of the present invention provides one or more of the aforementioned fusion proteins of the invention, for use in the prevention, treatment or suppression of CDI (eg. in a mammal such as man). Put another way, the present invention provides a method for the prevention, treatment or suppression of CDI (eg. in a mammal such as man), said method comprising administration of a therapeutically effective amount of one or more of the aforementioned fusion proteins of the invention to a subject (eg. a mammal such as man).

By way of example, a Toxin A-based fusion protein (any A toxinotype) may be employed alone or in combination with a Toxin B-based fusion protein (any B toxinotype). Similarly, a Toxin B-based fusion protein (any B toxinotype) may be employed alone or in combination with a Toxin A-based fusion protein (any A toxinotype). Said fusion proteins may be administered in a sequential or simultaneous manner. Vaccine applications of the present invention may further include the combined use (e.g. prior, sequential or subsequent administration) of one or more antigens such as a *C. difficile* antigen (e.g. a non-Toxin antigen; or a *C. difficile* bacterium such as one that has been inactivated or attenuated), and optionally one or more nosocomial infection antigens (e.g. an antigen, notably a surface antigen, from a bacterium that causes nosocomial infection; and/ or a bacterium that causes a nosocomial infection such as one that has been inactivated or attenuated). Examples of bacteria that cause nosocomial infection include one or more of: E. coli, Klebsiella pneumonae, Staphylococcus aureus such as MRSA, Legionella, Pseudomonas aeruginosa, Serratia marcescens, Enterobacter spp, Citrobacter spp, Stenotrophomonas maltophilia, Acinetobacter spp such as Acinetobacter baumannii, Burkholderia cepacia, and Enterococcus such as vancomycin-resistant Enterococcus (VRE).

In one embodiment, said vaccine application may be employed prophylactically, for example to treat a patient before said patient enters a hospital (or similar treatment facility) to help prevent hospital-acquired infection. Alternatively, said vaccine application may be administered to vulnerable patients as a matter of routine.

A related vaccine aspect of the invention provides one or more antibodies (comprising or consisting whole IgG and/or Fab and/or F(ab')2 fragments) that binds to the one or more

aforementioned fusion proteins of the invention, for use in the prevention, treatment or suppression of CDI (eg. in a mammal such as man). Put another way, the present invention provides a method for the prevention, treatment or suppression of CDI (eg. in a mammal such as man), said method comprising administration of a therapeutically effective amount of said antibody (or antibodies) to a subject (eg. a mammal such as man).

By way of example, an anti-Toxin A-based fusion protein (any A toxinotype) antibody may be employed alone or in combination with an anti-Toxin B-based fusion protein (any B toxinotype).antibody. Similarly, an anti-Toxin B-based fusion protein (any B toxinotype) antibody may be employed alone or in combination with an anti-Toxin A-based fusion protein (any A toxinotype) antibody. Said antibodies may be administered in a sequential or simultaneous manner. Vaccine applications of the present invention may further include the combined use (e.g. prior, sequential or subsequent administration) of one or more antibodies that bind to antigens such as a *C. difficile* antigen (e.g. a non-Toxin antigen; or a *C. difficile* bacterium), and optionally one or more antibodies that bind to one or more nosocomial infection antigens (e.g. an antigen, notably a surface antigen, from a bacterium that causes nosocomial infection; and/ or a bacterium that causes a nosocomial infection). Examples of bacteria that cause nosocomial infection include one or more of: E. coli, Klebsiella pneumonae, Staphylococcus aureus such as MRSA, Legionella, Pseudomonas aeruginosa, Serratia marcescens, Enterobacter spp, Citrobacter spp, Stenotrophomonas maltophilia, Acinetobacter spp such as Acinetobacter baumannii, Burkholderia cepacia, and Enterococcus such as vancomycin-resistant Enterococcus (VRE).

In one embodiment, said vaccine application may be employed prophylactically, for example once a patient has entered hospital (or similar treatment facility). Alternatively, said vaccine application may be administered to patients in combination with one or more antibiotics.

In one embodiment, said antibodies have been generated by immunisation of an animal (eg. a mammal such as man, or a non-human animal such as goat or sheep) with one or more of the aforementioned fusion proteins of the present invention.

In one embodiment, the antibodies of the present invention do not (substantially) bind to the effector domain and/ or to the cysteine protease domain of a *C. difficile* Toxin A and/ or Toxin B.

For the preparation of vaccines for human (or non-human animal) use, the active immunogenic ingredients (whether these be antigenic fusion protein/s of the present

invention and/ or corresponding antibodies of the invention that bind thereto) may be mixed with carriers or excipients, which are pharmaceutically acceptable and compatible with the active ingredient. Suitable carriers and excipients include, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

The vaccine may further comprise one or more adjuvants. One non-limiting example of an adjuvant with the scope of the invention is aluminium hydroxide. Other non-limiting examples of adjuvants include but are not limited to: N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2- (1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2 % squalene/ Tween 80 emulsion.

Typically, the vaccines are prepared as injectables, either as liquid solutions or suspensions. Of course, solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the peptide encapsulated in liposomes or microcapsules.

Vaccine administration is generally by conventional routes *e.g.* intravenous, subcutaneous, intraperitoneal, or mucosal routes. The administration may be by parenteral injection, for example, a subcutaneous or intramuscular injection.

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be particular to each subject.

The vaccine may be given in a single dose schedule, or optionally in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be

with 1-6 separate doses, followed by other doses given at subsequent time intervals required to maintain and /or reinforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual and be dependent upon the judgment of the practitioner.

In addition, the vaccine containing the immunogenic antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, immunoglobulins, antibiotics, interleukins (*e.g.*, IL-2, IL-12), and/or cytokines (*e.g.*, IFN gamma)

Additional formulations suitable for use with the present invention include microcapsules, suppositories and, in some cases, oral formulations or formulations suitable for distribution as aerosols. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5 % to 10 %, including for instance, about 1 %-2 %.

Fusion proteins of the invention may also have uses as ligands for use in affinity chromatography procedures. In such procedures, fusion proteins of the invention may be covalently immobilised onto a matrix, such as Sepharose, e.g. using cyanogen bromide-activated Sepharose. Such affinity columns may then be used to purify antibody from antisera or partially purified solutions of immunoglobulins by passing them through the column and then eluting the bound IgG fraction (e.g. by low pH). Almost all of the antibody in the eluted fraction will be directed against the fusion proteins of the invention, with non-specific antibodies and other proteins having been removed. These affinity purified IgG fractions have applications both as immunotherapeutics and as reagents in diagnostics. For immunotherapeutics, affinity purified antibodies enable a lower dose to be administered making adverse side effects less likely. For diagnostics, affinity purified agents often give improved specificity and fewer false positive results.

Definitions Section

Clostridium difficile is a species of Gram-positive bacterium of the genus Clostridium.

Clostridium difficile infection (CDI) means a bacterial infection which affects humans and animals and which results in a range of symptoms from mild self-limiting diarrhoea to life-threatening conditions such as pseudomembranous colitis and cytotoxic megacolon. In this disease, *C. difficile* replaces some of the normal gut flora and starts to produce cytotoxins

which attack and damage the gut epithelium. Primary risk factors for human CDI include: receiving broad-spectrum antibiotics, being over 65 years old and being hospitalised.

Clostridium difficile Toxin A is a family of protein cytotoxins/ enterotoxins of approximately 300 kDa in size. Toxin A has an enzyme activity within the N-terminal region which acts to disrupt the cytoskeleton of the mammalian cell causing cell death. There a number of naturally occurring variants of Toxin A within the strains of *Clostridium difficile* which are called 'toxinotypes'. The various toxinotypes of Toxin A have variations within their primary sequence of usually <10% overall. Examples of suitable Toxin A sequences include SEQ ID NOs: 1 and 3.

Clostridium difficile Toxin B is a family of protein cytotoxins of approximately 270 kDa in size which are similar to Toxin A but significantly more cytotoxic. Like Toxin A, Toxin B has an enzyme activity within the N-terminal region which acts to disrupt the cytoskeleton of the mammalian cell causing cell death. There are a number of naturally occurring variants of Toxin B within the strains of *C. difficile* which are called 'toxinotypes'. The various toxinotypes of Toxin B have variations within their primary sequence of up to 15% overall. Examples of suitable Toxin B sequences include SEQ ID NOs: 2 and 4.

C. difficile repeat units are regions within the C-terminus of Toxin A and B that contain repeating motifs which were first identified by von Eichel-Streiber and Sauerborn (1990; Gene 30: 107-113). In the case of Toxin A there are 31 short repeats and 7 long repeats with each repeat consisting of a β-hairpin followed by a loop. Toxin B consists of a similar structure but with fewer repeats. The repeat units of Toxin A are contained within residues 1850 -2710 and those for Toxin B within residues 1852 -2366. The repeat regions play a role in receptor binding. The receptor binding regions (i.e. that define the toxin's structural binding pockets) appear to be clustered around the long repeat regions to form 'binding modules' (see Tables 1 and 2).

Central domains of Toxin A and B are believed to play a role in translocation of the toxins into mammalian cells. The central domains of Toxin A are based on residues 543-1849 and those for Toxin B are based on residues 543-1851. Of the central domain regions of Toxins A and B, the first domain is a cysteine protease, which plays a role in the internalisation of the toxin's effector domain (which contains the glucosyl transferase activity).

Toxinotypes are often used to classify strains of *C. difficile*. Toxinotyping is based on a method which characterises the restriction patterns obtained with the toxin genes.

Toxinotypes of Toxins A and B represent variants, by primary amino acid sequence, of these protein toxins. In one embodiment, the *C. difficile* toxin is selected from one of toxinotypes 0 to XV. Preferred Toxinotypes (plus example Ribotypes and Strains) are listed in the Table immediately below. The listed Toxinotypes are purely illustrative and are not intended to be limiting to the present invention

Toxinotype	Example Ribotypes	Example Strains	Reference
0	001, 106	VPI10463	Rupnik et al. (1998) J. Clinical Microbiol. 36: 2240-2247
1	003, 012, 102	EX623	
2	103	AC008	
3	027, 034, 075, 080	R20291, QCD-32g58	
4	023, 034, 075, 080	55767	
5	066, 078	SE881	
6	045, 063, 066	51377	
7	063	57267	
8	017, 047	1470	
9	019	51680	
10	036	8864	
11	033	IS58, R11402	Rupnik et al. (2001) Microbiology 147: 439-447
12	056	IS25	
13	070	R9367	
14	111	R10870	
15	122	R9385	

An "antibody" is used in the broadest sense and specifically covers polyclonal antibodies and antibody fragments so long as they exhibit the desired biological activity. For example, an antibody is a protein including at least one or two, heavy (H) chain variable regions (abbreviated herein as VHC), and at least one or two light (L) chain variable regions (abbreviated herein as VLC). The VHC and VLC regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" (FR). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E.A., et al. Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, 1991, and Chothia, C. et al, J. Mol. Biol.

196:901-917, 1987. Preferably, each VHC and VLC is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FRI, CDRI, FR2, CDR2, FR3, CDR3, FR4.

The VHC or VLC chain of the antibody can further include all or part of a heavy or light chain constant region. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. The heavy chain constant region includes three domains, CHI, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda.

The term antibody, as used herein, also refers to a portion of an antibody that binds to a toxin of *C. difficile* (e.g. Toxin A or B), e.g., a molecule in which one or more immunoglobulin chains is not full length, but which binds to a toxin. Examples of binding portions encompassed within the term antibody include (i) a Fab fragment, a monovalent fragment consisting of the VLC, VHC, CL and CHI domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fc fragment consisting of the VHC and CHI domains; (iv) a Fv fragment consisting of the VLC and VHC domains of a single arm of an antibody, (v) a dAb fragment (Ward et al, Nature 341:544-546, 1989), which consists of a VHC domain; and (vi) an isolated complementarity determining region (CDR) having sufficient framework to bind, e.g. an antigen binding portion of a variable region. An antigen binding portion of a light chain variable region and an antigen binding portion of a heavy chain variable region, e.g., the two domains of the Fv fragment, VLC and VHC, can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VLC and VHC regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science IAI-ATi-Aiβ; and Huston et al. (1988) Proc. Natl. Acad. ScL USA 85:5879-5883). Such single-chain antibodies (as well as camelids) are also encompassed within the term antibody. These are obtained using conventional techniques known to those with skill in the art, and the portions are screened for utility in the same manner as are intact antibodies.

The term "fragment" means a peptide typically having at least seventy, preferably at least eighty, more preferably at least ninety percent of the consecutive amino acid sequence of the reference sequence.

The term "variant" means a peptide or peptide fragment having at least eighty, preferably at least eighty five, more preferably at least ninety percent amino acid sequence homology with a *C. difficile* toxin polypeptide. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences may be compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequent coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percentage sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, e.g., segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art. Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, e.g., CLUSTAL W, see, e.g., Julie D. Thompson et al., CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position- Specific Gap Penalties and Weight Matrix Choice, 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, e.g., Osamu Gotoh, Significant Improvement in Accuracy of Multiple Protein. Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments, 264(4) J. Mol. Biol. 823-838 (1996). Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, e.g., Match-box, see, e.g., Eric Depiereux and Ernest Feytmans, Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences, 8(5) CABIOS 501 -509 (1992); Gibbs sampling, see, e.g., C. E. Lawrence et al., Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment, 262(5131) Science 208-214 (1993); Align-M, see, e.g., Ivo Van Walle et al., Align-M - A New Algorithm for Multiple Alignment of Highly Divergent Sequences, 20(9) Bioinformatics:1428-1435 (2004).

Thus, percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-19, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown below (amino acids are indicated by the standard one-letter codes).

Alignment scores for determining sequence identity

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{[\text{length of the longer sequence plus the number of gaps introduced into the longer}} \times 100$$

sequence in order to align the two sequences]

Substantially homologous polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see below) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag.

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan
	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

In addition to the 20 standard amino acids, non-standard amino acids (such as 4-hydroxyproline, 6-*N*-methyl lysine, 2-aminoisobutyric acid, isovaline and α -methyl serine) may be substituted for amino acid residues of the polypeptides of the present invention. A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, and unnatural amino acids may be substituted for clostridial polypeptide amino

acid residues. The polypeptides of the present invention can also comprise non-naturally occurring amino acid residues.

Non-naturally occurring amino acids include, without limitation, trans-3-methylproline, 2,4-methano-proline, cis-4-hydroxyproline, trans-4-hydroxy-proline, N-methylglycine, allo-threonine, methyl-threonine, hydroxy-ethylcysteine, hydroxyethylhomo-cysteine, nitro-glutamine, homoglutamine, pipecolic acid, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenyl-alanine, 4-azaphenyl-alanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in Xenopus oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the polypeptdie in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for amino acid residues of polypeptides of the present invention.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989). Sites of biological interaction can also be determined by physical analysis of structure, as determined

by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-12, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992; Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related components (e.g. the translocation or protease components) of the polypeptides of the present invention.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-7, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-6, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenised polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-7, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Toxin-neutralising means the capacity of a substance to prevent the cytotoxic action of either Toxin A or B on a mammalian cell. In assays for toxin-neutralising activity, a fixed amount of toxin is mixed with various concentrations of a neutralising substance (e.g. an antibody) and the mixture applied to and incubated with a mammalian cell line (e.g. Vero cells) for a fixed time. The dilution of the substance (antibody) that completely protects the cells from the cytotoxic effects of either Toxin A or B (evident by cell rounding) may be defined as the neutralising titre.

FIGURES

Figure 1 illustrates to structures of *C. difficile* Toxins A and B showing amino acid residues at the various domain boundaries.

Figure 2 illustrates TxB3 purification. The left-hand Figure shows a 4-12 % SDS-PAGE analysis of TxB3. M1 = SeeBlue® Plus2 Pre-Stained Standard, M2 = MagicMark™ XP Standard. The right-hand Figure shows a Western blot analysis of TxB3 with ovine anti-TcdB polyclonal antibodies. M1 and M2 are as described for the left-hand Figure.

Figure 3 illustrates TxB4 purification. The left-hand Figure shows a 4-12 % SDS-PAGE analysis of TxB4. M = SeeBlue® Plus2 Pre-Stained Standard. The right-hand Figure shows a Western blot analysis of TxB4 with ovine anti-TcdB polyclonal antibodies, M = MagicMark™ XP Standard.

Figure 4 illustrates TxB5 purification. The left-hand Figure shows a 4-12 % SDS-PAGE analysis of TxB5. M = SeeBlue® Plus2 Pre-Stained Standard. The right-hand Figure shows a Western blot analysis of TxB5 with ovine anti-TcdB polyclonal antibodies, M = MagicMark™ XP Standard.

Figure 5 illustrates TxA4 purification and SDS-PAGE analysis of the nickel affinity purification of HRV3C protease treated TxA4. M= Molecular weight markers, L = column load, A8 = column flow-through. Fractions A14 – B14 showed the purified TxA4.

EXAMPLES

Example 1 – Cloning and expression of antigens derived from Toxins A and B

Genes encoding these peptides may be made commercially with codon bias for any desired expression host (e.g. *E. coli*, *Pichia pastoris*). Peptides are expressed from these genes using standard molecular biology methods (e.g. Sambrook et al. 1989, Molecular Cloning a Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). One convenient method of cloning is the Gateway ® system (Invitrogen) which allow genetic constructs to be assembled in a modular fashion.

Protocol 1: The Gateway LR recombination reaction – a general protocol

Materials: Antigen gene (Toxin A or B)) entry clones were synthesised by Entelechon. Gateway ® LR Clonase ™ II Enzyme Mix was purchased from Invitrogen. Gateway ® Nova pET Destination vectors were purchased from Calbiochem Nova, part of Merck Chemicals Ltd.

Toxin A or B entry clone (1 µl), destination vector (1 µl) and TE buffer (6 µl) were mixed at room temperature in a 1.5 ml microcentrifuge tube. LR Clonase ™ II was placed on ice for two minutes and mixed briefly with vortexing (2 x 2 s). The clonase enzyme (2 µl) was added to the microcentrifuge tube and the components mixed with gentle pipetting. Recombinations were incubated at 25 °C for 1 hour. Proteinase K solution (1 µl, 2 µg / µl) was added and the

reactions incubated at 37 °C for 10 minutes. The resultant solution (1 µl) was used to transform chemically competent *E. coli*.

Protocol 2: Transformation of chemically competent cells – a general protocol
Materials: OneShot® BL21 Star™ (DE3) and One Shot® TOP10 chemically competent *E. coli* and SOC media were purchased from Invitrogen. Ampicillin was purchased from Sigma Aldrich.

LR recombination reaction or plasmid DNA (1 µl) was pipetted into an aliquot (50 µl) of BL21 Star™ or TOP10 chemically competent *E. coli*. The mixture was incubated on ice for 30 minutes and subsequently heat shocked in a water bath at 42 °C for 30 seconds. The cell aliquot was returned to the ice and SOC media (250 µl) added. Transformations were maintained in SOC media at 37 °C for 1 hour with orbital shaking (180 rpm). Transformation culture (100 – 200 µl) was plated out onto LB agar supplemented with ampicillin (100 µg / ml). The plates were incubated at 37 °C for 15 minutes, inverted and maintained at the same temperature overnight.

Example 2 – Purification of antigens of the invention - expression and purification of *C. difficile* Toxin B fragment TxB3
Toxin B-derived antigen TxB3(-h) (eg. Seq ID 9) was expressed as a thioredoxin fusion protein (Seq ID 27).

An N-his_6-thioredoxin fusion of TxB3 was expressed in BL21 Star™ (DE3) *E. coli* harbouring plasmid pDest59TxB3. LB media (3 x 20 ml) supplemented with 100 µg / ml ampicillin and 0.5 % glucose was inoculated from a glycerol cell stock (cell culture < OD_{600} 1 [500 µl] + glycerol [125 µl]). Cultures were maintained at 37 °C for 6-7 hours with orbital shaking (180 rpm). Each culture was used to inoculate LB media (100 ml) supplemented with 100 µg / ml ampicillin and 0.5 % glucose. Cultures were maintained at 37 °C for 1 hour with orbital shaking (180 rpm). Terrific Broth (3 x 1 L) supplemented with 100 µg / ml ampicillin and 0.1 % glucose was inoculated with the LB culture (100 ml per litre) and maintained at 37 °C as before to an absorbance at 600 nm of 0.5. Expression was induced with the addition of IPTG to a final concentration of 1 mM and the cultures maintained at 16 °C overnight with orbital shaking (180 rpm). Cells were harvested by centrifugation for 30 minutes (3000 rpm, Sorvall RC3BP centrifuge, rotor # H6000A), resuspended in low imidazole buffer

(100 ml, pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 20 mM imidazole) and frozen at -80 °C.

(i) Nickel affinity purification of the thioredoxin TxB3 fusion protein

Cell paste was thawed at room temperature and then on ice until liquefied. Cells were disrupted with sonication (10 cycles of 30 s ON and 30 s OFF) and the resultant lysate cleared by centrifugation for 30 minutes (14,000 rpm, Sorvall RC5C centrifuge, rotor #SS-34). Cleared lysate was applied to fast flow chelating sepharose charged with nickel ions (40 ml bed volume) at a flow rate of 1 ml / min. The column was washed with low imidazole buffer (pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 20 mM imidazole) until the absorbance of the flow through at 280 nM returned to near baseline levels. Bound material was eluted with sequential steps to 15, 25 and 70 % high imidazole buffer (pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 500 mM imidazole). Material eluted at 70 % high imidazole buffer was pooled and dialysed into thrombin cleavage buffer (20 mM Tris-HCl pH 8.4, 150 mM sodium chloride, 2.5 mM calcium chloride) overnight.

(ii) Thrombin digestion of the thioredoxin TxB3 fusion protein

Human thrombin (Novagen, 1U per mg of total protein) was added to the pooled nickel column fractions which had been dialysed into thrombin cleavage buffer. The digest was incubated at 25 °C for 4 hours and frozen at -80 °C to prevent continued cleavage.

(iii) Nickel affinity purification of TxB3

The thrombin digest was thawed on ice and p-Aminobenzamidine resin added (0.1 ml drained resin per 6U of thrombin). The mixture was gently rocked over ice for 30 minutes and the resin filtered off. The cleared filtrate was passed over fast flow chelating sepharose charge with nickel ions (6 ml bed volume) at a flow rate of 1 ml / min and the flow through pooled and dialysed into storage buffer (pH 7.4, 50 mM HEPES, 150 mM sodium chloride). The solution was sterile filtered into 1 ml aliquots. The total protein obtained was 10.5 mg, which was estimated to be 55 % TxB3. Protein was also analysed by Western blotting with ovine anti-TcdB polyclonal antibodies (**Figure 2**).

Example 3 – Purification of antigens of the invention - expression and purification of *C. difficile* Toxin B fragment TxB4

Large scale expression of the Nus TxB4 fusion protein

A bead from a -80 °C stock of BL21 Star (DE3) *E. coli* harbouring plasmid pDest57TxB4His was streaked onto L-agar supplemented with 100 µg / ml ampicillin and incubated at 37 °C overnight. A single colony was used to inoculate 2YT media (100 ml) supplemented with 100 µg / ml ampicillin and 0.5 % glucose. The culture was maintained at 37 °C with orbital shaking (180 rpm) to an absorbance of 0.6 at 600 nm and used as a 5 % inoculum for Terrific Broth (2 x 1L) supplemented with 100 µg / ml ampicillin and 0.5 % glucose. Cultures were maintained as before to an absorbance of 0.6 at 600 nm and the temperature lowered to 16 °C. Protein expression was induced by the addition of IPTG to a final concentration of 1 mM following thermal equilibration and the culture maintained overnight. Cells were harvested by centrifugation for 30 minutes (3000 rpm, Sorvall RC3BP centrifuge, rotor # H6000A), resuspended in low imidazole buffer (1:4 cell paste to buffer w / v, 50 mM HEPES pH 7.4, 500 mM sodium chloride, 20 mM imidazole) and frozen at -80 °C.

Nickel affinity purification of the Nus TxB4 fusion protein

Cell paste was thawed at room temperature and then on ice until liquefied. Cells were disrupted with sonication (15 cycles of 30 s ON and 30 s OFF) and the resultant lysate cleared by centrifugation for 30 minutes at 4 °C (16,000 rpm, Sorvall RC5C centrifuge, rotor # SS-34). Cleared lysate was applied to fast flow chelating sepharose charged with nickel ions (40 ml bed volume) at a flow rate of 2 ml / min. The column was washed with low imidazole buffer (pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 20 mM imidazole) until the absorbance of the flow through at 280 nm returned to near baseline levels. Bound material was eluted with a step to 50 % high imidazole buffer (pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 500 mM imidazole). Material eluted from the column was analysed by SDS-PAGE and selected fractions pooled. Protein concentration was determined from the absorbance at 280 nm.

Thrombin digestion of the Nus TxB4 fusion protein

To each 10 µg of protein, 5 µl of thrombin digest buffer (200 mM Tris-HCl pH 8.4, 1.5 M NaCl, 25 mM $CaCl_2$), 1 µl of Human thrombin (Novagen) diluted 200 fold in thrombin dilution buffer (50 mM sodium citrate, pH 6.5, 200 mM NaCl, 0.1% PEG-8000, 50% glycerol) and water to a volume of 50 µl were added. The protein was digested overnight at room temperature and dialysed into low imidazole buffer at 4°C.

Nickel affinity purification of TxB4

TxB4 in low imidazole buffer was applied to fast flow chelating sepharose charged with nickel ions (40 ml bed volume) at a flow rate of 3 ml / min. The column was washed with low imidazole buffer until the absorbance of the flow through at 280 nm returned to near baseline levels. The column was washed with 80 mM imidazole, protein eluting after the resultant initial peak in UV absorbance (280 nm) was collected and dialysed into storage buffer (50 mM HEPES pH 7.4, 150 mM sodium chloride). Protein was analysed by SDS-PAGE and Western blotting with ovine anti-TcdB polyclonal antibodies (**Figure 3**).

Example 4 - Expression and purification of *C. difficile* Toxin B fragment TxB5 (residues 544-2366 of Toxin B)

Large scale expression of the Nus TxB5 fusion protein

An N-his_6-Nus fusion of TxB5 was expressed in BL21 Star™ (DE3) *E. coli* harbouring plasmid pDest57TxB5. An overnight culture in LB media supplemented with 100 µg / ml ampicillin was used as a 3 % inoculum for Terrific Broth (3L) supplemented with 100 µg / ml ampicillin. Cultures were maintained at 37 °C to an absorbance at 600 nm of 0.6 with orbital shaking (180 rpm). Expression was induced with the addition of IPTG to a final concentration of 1 mM and the cultures maintained at 16 °C overnight with orbital shaking (180 rpm). Cells (25 g) were harvested by centrifugation for 30 minutes (3000 rpm, Sorvall RC3BP centrifuge, rotor # H6000A) and resuspended in low imidazole buffer (250 ml, pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 20 mM imidazole).

Nickel affinity purification of the Nus TxB5 fusion protein

Lysozyme (10 mg) was added to the resuspended cells and the mixture stirred for 15 minutes. Cells were disrupted with sonication (10 cycles of 30 s ON and 30 s OFF)

and the resultant lysate cleared by centrifugation for 30 minutes (14,000 rpm, Sorvall RC5C centrifuge, rotor #SS-34). Half of the cleared lysate was applied to fast flow chelating sepharose charged with nickel ions (40 ml bed volume) at a flow rate of 2 ml / min. The column was washed with low imidazole buffer until the UV absorbance of the flow through at 280 nm returned to near baseline levels. Protein, including Nus TxB5, was eluted with a step to 38 % (200 mM imidazole) high imidazole buffer (pH 7.4, 50 mM HEPES, 500 mM sodium chloride, 500 mM imidazole). The second half of the lysate was processed in the same manner and the pooled eluted protein dialysed overnight into high salt HIC buffer (pH 7.4, 50 mM HEPES, 750 mM ammonium sulphate).

Butyl-s hydrophobic interaction chromatography purification of Nus TxB5

Half of the pooled protein solution in high salt HIC buffer was applied to a column containing butyl-s-sepharose 6 fast flow resin (9 ml bed volume). The column was washed with high salt HIC buffer (pH 7.4, 50 mM HEPES, 750 mM ammonium sulphate) until the UV absorbance of the flow through at 280 nm returned to near baseline levels. Protein was eluted from the column with a step to 100 % low salt HIC buffer ((pH 7.4, 50 mM HEPES). The other half of the protein from the first nickel column was purified in the same manner. The eluted protein was pooled in preparation for digestion with thrombin.

Thrombin digestion of the Nus TxB5 fusion protein

Pooled protein from the HIC column (69 mg, 30 ml) was added to a solution containing 10x thrombin cleavage buffer (15 ml, 200 mM Tris-HCl pH 8.4, 1.5 M sodium chloride, 25 mM calcium chloride), deionised water (105 ml) and human thrombin (Novagen, 40 U). The solution was incubated at room temperature for 4 hours and PMSF added to a final concentration of 1 mM. The resultant protein including the TxB5 was dialysed into high salt HIC buffer.

Butyl-s hydrophobic interaction chromatography purification of TxB5

The TxB5 from the thrombin digest was purified in two batches. Each batch was applied in high salt HIC buffer (pH 7.4, 50 mM HEPES, 750 mM ammonium sulphate) to a column containing butyl-s-sepharose 6 fast flow resin (9 ml bed volume) at a flow rate of 1 ml / min. The column was washed with high salt HIC

buffer until the UV absorbance of the flow through at 280 nm returned to near baseline levels. Protein was eluted from the column with a step to 100 % low salt HIC buffer (pH 7.4, 50 mM HEPES). The eluted material was dialysed against buffer (pH 7.4, 50 mM HEPES) overnight.

Q sepharose ion exchange chromatography purification of TxB5

The TxB5 in buffer (pH 7.4, 50 mM HEPES) was run through a column containing Q sepharose fast flow resin (5 ml bed volume) at a flow rate of 1 ml / min. The flow through was pooled and dialysed into storage buffer (pH 7.4, 50 mM HEPES, 150 mM sodium chloride). Approximately 20 mg of protein was produced, of this 60 % was TxB5 based on SDS-PAGE analysis. The protein was frozen in 1 ml aliquots at -80 °C. Protein was analysed by SDS-PAGE and Western blotting with ovine anti-TcdB polyclonal antibodies (**Figure 4**).

Example 5 - Expression and purification of *C. difficile* Toxin A fragment TxA4 (residues 770-2710 of Toxin A)

Expression

L-broth (100 ml) supplemented with 50 µg/ml kanamycin and 0.2 % glucose was inoculated with a scrape from a glycerol freeze (BL21 (DE3) *E. coli* harbouring plasmid pET28aHis$_6$TrxHRV3CαNaturalTxA4) and maintained overnight at 30°C and 180 rpm. The overnight culture was used as a 2 % inoculum for Terrific Broth (4 x 0.5 L in 2L unbaffled flasks) supplemented with 50 µg/ml kanamycin and 0.2 % glucose. Cultures were maintained at 37°C with orbital shaking (180 rpm) to an absorbance at 600 nm of 0.6. The temperature of the cultures was reduced to 16°C and protein expression induced with the addition of 1 mM IPTG. The culture was maintained overnight at 16°C with orbital shaking as before. Cell paste (23 g) was harvested by centrifugation (Sorvall RC3BP centrifuge, H6000A rotor, 4000 g, 20 minutes). The paste was recovered from the centrifuge pots by resuspension in low imidazole buffer (pH 7.5, 50 mM Hepes, 0.5 M sodium chloride, 20 mM Imidazole) and stored at -80°C.

Immobilised nickel affinity purification of the TxA4 precursor

Cells (23g) resuspended with 85 ml of low imidazole buffer (pH7.5, 50 mM Hepes, 0.5 M NaCl 20 mM imidazole) was subjected to lysis using sonication. The lysate was cleared by centrifugation (Sorvall RC5C centrifuge, SS-34 rotor, 20,000 g, 20 minutes) and applied to a 20 ml nickel column (Ø 26 mm) at a flow rate of 1.5 ml/min. The column was washed with

ten column volumes of low imidazole buffer and bound protein eluted using a five column volume gradient to 100 % high imidazole buffer (pH7.5, 50 mM Hepes, 0.5 M NaCl, 0.5 M imidazole). Fractions were analysed on 4-12% NuPAGE Bis-Tris polyacrylamide gels with coomassie staining.

Cleavage of the fusion partner and His_6-tag

The purest fractions were pooled and dialysed against HRV3C cleavage buffer (2L, pH 7.5, 20 mM Tris-HCl, 0.5 M NaCl) overnight at 4°C. HRV3C protease (10 U per mg of full length target protein) was added to the solution and incubated at 20°C for five hours followed by 4°C overnight.

Immobilised nickel affinity purification of post cleavage TxA4

The protein solution (pH 7.5 20 mM Tris-HCl, 0.5 M NaCl) was passed over a 20 ml nickel column (Ø 26 mm) at a flow rate of 1.5 ml/min. Some protein was seen to elute in the flow through as judged by the UV absorbance. The column was given a short wash with the HRV3C cleavage buffer and the TxA4 eluted with 5 % high imidazole buffer (pH7.5, 50 mM Hepes, 0.5 M NaCl, 0.5 M imidazole) at an imidazole concentration of 25 mM. The remaining proteins were eluted from the column with a four column volume gradient to 100 % high imidazole buffer. The purest fractions were pooled and dialysed into storage buffer (pH 7.5 50 mM Hepes, 0.5 M NaCl). Fractions from the final purification colum are shown in Figure 5.

Example 6 - Expression and purification of *C. difficile* Toxin A fragment TxA4 truncated (residues 770-2389 of Toxin A)

Expression

L-Broth (100 ml) supplemented with 100 µg/ml ampicillin and 0.5% glucose was inoculated with a colony (harbouring pET59His6TRXtcsαnaturalTxA4truncate) from an overnight growth on a L-agar plate supplemented with 100 µg/ml ampicillin and maintained overnight at 37°C and 180 rpm. This was used as an inoculum for Terrific Broth (6 x 1000 mls in 2000ml unbaffled flasks) supplemented with 100 µg/ml ampicillin and 0.5% glucose. Cultures were maintained at 37°C with orbital shaking (180 rpm) to an absorbance at 600 nm of 0.6. The temperature of the cultures was reduced to 16°C and protein expression induced with the addition of IPTG to a final concentration of 1 mM. The culture was maintained overnight at 16°C with orbital shaking as before. Cell paste was harvested by centrifugation (Sorvall RC3BP

centrifuge, H6000A rotor, 4000g, 30 minutes). The paste was recovered from the centrifuge pots by re-suspension in Hepes buffer (50 mM Hepes pH 7.4, 0.5 M sodium chloride) and stored at -20°C.

Immobilsed nickel affinity purification of the TxA4 truncate precursor

Cells (44 g) re-suspended with 180 ml of Hepes buffer (50 mM Hepes pH 7.4, 500 mM NaCl) were subject to lysis using sonication. The lysate was clarified by centrifugation at 4000 rpm for 20 minutes (Heraeus Multifuge). The supernatant was retained and applied to a 64 ml Zinc Sepharose column (XK26 x 12) at a flow rate of 5 ml/minute. The column was washed until the absorbance at 280 nm was reduced to the baseline. The bound protein was eluted using a gradient of 0 – 250 mM imidazole in 50 mM Hepes pH 7.4, 500 mM sodium chloride. The fractions were analysed on 4-12% NuPAGE Bis-Tris polyacrylamide gels with coomassie staining.

Cleavage of the fusion partner and His_6 – tag

The purest fractions were pooled and dialysed against thrombin cleavage buffer (20 mM Tris/HCl pH 8.4 + 150 mM NaCl + 2.5 mM Ca Cl_2) overnight at +4°C. Restriction grade thrombin (Novagen) was added at 1:2000 wt: wt with respect to the target protein. The mixture was incubated at room temperature overnight.

Immobilised zinc affinity purification of post cleavage TxA4 truncate

The protein solution (in 50 mM Hepes pH 7.4, 500 mM sodium chloride) was passed over a 24 ml zinc column (XK16 x 12) at a flow rate of 2 ml/minute. The column was washed with equilibration buffer (50 mM Hepes pH 7.4, 500 mM sodium chloride) until the absorbance at 280 nm was reduced to the baseline. The bound protein was eluted using a gradient of 0 – 250 mM imidazole in 50 mM Hepes pH 7.4, 500 mM sodium chloride.

Example 7 – Formulation of antigens of the invention for immunisation of animals

Purified *C. difficile* antigens at a concentration of between 0.5 – 2 mg/ml (nominally 1 mg/ml) were dialysed against a suitable buffer (e.g. 10mM Hepes buffer pH 7.4 containing 150mM NaCl) and then formaldehyde added to a final concentration of 0.2% and incubated for up to 7 days at 35°C. After incubation, the formaldehyde

may optionally be removed by dialysis against a suitable buffer, e.g. phosphate buffered saline.

For sheep, 2 ml of buffer solution containing between 10 and 500 µg of the above *C. difficile* antigen is mixed with 2.6 ml of Freund's adjuvant to form an emulsion. Mixing with the adjuvant is carried out for several minutes to ensure a stable emulsion. The complete form of the adjuvant is used for the primary immunisation and incomplete Freund's adjuvant for all subsequent boosts.

Example 8 – Generation of antibodies to antigens of the invention

A number of conventional factors are taken into consideration during the preparation of antiserum in order to achieve the optimal humoral antibody response. These include: breed of animal; choice of adjuvant; number and location of immunisation sites; quantity of immunogen; and number of and interval between doses. With conventional optimisation of these parameters is routine to obtain specific antibody levels in excess of 6 g/litre of serum.

For sheep, an emulsion of the antigen with Freund's adjuvant was prepared as described as in Example 7. The complete form of the adjuvant is used for the primary immunisation and incomplete Freund's adjuvant for all subsequent boosts. About 4.2 ml of the antigen/adjuvant mixture was used to immunise each sheep by i.m. injection and spread across 6 sites including the neck and all the upper limbs. This was repeated every 28 days. Blood samples were taken 14 days after each immunisation.

For comparison of the toxin-neutralising immune response to the different antigens, 3 sheep were used per antigen. They were immunised as above using an identical protocol and the same protein dose per immunisation.

Example 9 – Assessment of the neutralising efficacy of antisera to toxins using the *in vitro* cell assay

The toxin neutralizing activity of the antisera against *C. difficile* Toxins was measured by cytotoxicity assays using Vero cells. A fixed amount of either purified *C. difficile* Toxin A or Toxin B was mixed with various dilutions of the antibodies, incubated for

30min at 37°C and then applied to Vero cells growing on 96-well tissue culture plates. Both Toxin A and B possess cytotoxic activity which results in a characteristic rounding of the Vero cells over a period of 24 - 72 h. In the presence of neutralising antibodies this activity is inhibited and the neutralising strength of an antibody preparation may be assessed by the dilution required to neutralise the effect of a designated quantity of either Toxin A or B.

Data demonstrating the neutralising activity of ovine antibody to various recombinant *C. difficile* Toxin B antigens are shown in Tables 3-6. In these experiments, various dilutions of ovine antibody were mixed with Toxin B at a final concentration of 0.5 ng/ml and incubated for 30min at 37°C and then applied to Vero cells as above and incubated at 37° and monitored over a period of 24 -72 h. The antibody dilutions which completely protect the cells against the cytotoxic effects of the Toxin B were calculated. Similar data for Toxin A-derived antigens are shown in tables 7-10

Collectively, the data in Tables 3 -10 show the superior capacity of fusion proteins of the invention to elicit a toxin-neutralising immune response compared to fragments containing just the repeat domains of either Toxin A or B.

Example 10 - Assessment of the *in vivo* efficacy of antiserum generated using recombinant antigens of the invention for treating CDI

To demonstrate the efficacy of the antisera generated, using recombinant antigens, to treat CDI in vivo, Syrian hamsters are passively immunised with antibodies which have neutralising activity against one or more of the toxins of *C. difficile*. For assessing the efficacy of a treatment formulation, hamsters will be given antibody either intravenously or by the intraperitoneal route at various times from 6 hours post-challenge to 240 hours post challenge with *C. difficile*

Prior to passively immunisation hamsters are administered a broad spectrum antibiotic (e.g. clindamycin) and 12-72 h later challenged with *C. difficile* spores by mouth. Animals are then monitored for up to 15 days for symptoms of *C. difficile*-associated disease. Control, non-immunised animals develop signs of the disease (e.g. diarrhoea, swollen abdomen, lethargy, ruffled fur) while those treated with ovine antibody appear normal.

Example 11- Vaccination by peptide/ peptide fragments of the invention

A vaccine, represented by a peptide/ peptide fragment of the invention is prepared by current Good Manufacturing Practice. Using such practices, peptides/ peptide fragments of the invention may be bound to an adjuvant of aluminium hydroxide which is commercially available (*e.g.* Alhydrogel). The vaccine would normally contain a combination of antigens of the invention derived from Toxin A and Toxin B but could also contain either Toxin A or B antigens. The vaccine may also contain Toxin A and B antigens in combination with other antigens of bacterial or viral origin.

Purified *C. difficile* Toxin A and/or Toxin B antigen of the invention may be treated with formaldehyde at a final concentration of 0.2% and incubated for up to 24 hours at 35°C (as described in Example 7). .

In addition to the antigens of the invention, a typical vaccine composition comprises:
A) A buffer (*e.g.*, Hepes buffer between 5 and 20 mM and pH between 7.0 and 7.5;
B) A salt component to make the vaccine physiologically isotonic (*e.g.* between 100 and 150 mM NaCl);
C) An adjuvant (*e.g.*, aluminium hydroxide at a final aluminium concentration of between 100 and 700µg per vaccine dose); and
D) A preservative (*e.g.*, Thiomersal at 0.01% or formaldehyde at 0.01%).

Such vaccine compositions are administered to humans by a variety of different immunisation regimens, such as:

1. A single dose (*e.g.*, 20 µg adsorbed fragment of the invention) in 0.5 ml administered sub-cutaneously.
2. Two doses (*e.g.*, of 10 µg adsorbed fragment of the invention) in 0.5 mls administered at 0 and 4 weeks.
3. Three doses (*e.g.*, of 10 µg adsorbed fragment of the invention) in 0.5 mls administered at 0, 2 and 12 weeks.

These vaccination regimens confer levels of protection against exposure to the homologous serotypes of *C. difficile* toxins

Example 12 - Affinity purification of IgG using immobilised constructs of the invention.

Preparation of the affinity chromatography medium

The construct of the invention to be immobilised is dialysed against a suitable coupling buffer e.g. 0.1 M $NaHCO_3$ pH 8.3 containing 0.5 M NaCl. Approximately 5 ml of protein solution at 1-3 mg/ml is added per ml of CNBr-activated Sepharose 4B powder. The mixture is rotated end-over end for 1 h at room temperature or overnight at 4 °C. Other gentle stirring methods may be employed. Excess ligand is then wash away excess with at least 5 medium (gel) volumes of coupling buffer. Any remaining active groups and then blocked. The medium is transferred to 0.1 M Tris-HCl buffer, pH 8.0 or 1 M ethanolamine, pH 8.0 and incubated 2 hours at room temperature. The gel is then washed with at least three cycles of alternating pH (at least 5 medium volumes of each buffer). Each cycle should consist of a wash with 0.1 M acetic acid/sodium acetate, pH 4.0 containing 0.5 M NaCl followed by a wash with. 0.1 M Tris-HCl, pH 8 containing 0.5 M NaCl. After washing the gel is transferred to a suitable storage buffer (e.g. 50mM HEPES pH 7.4 containing 0.15M NaCl and stored at 4° C until use

Purification of IgG

Affinity columns are prepared as above using antigens of the invention derived from either Toxin A or B. For purification of antibodies to Toxin B, a construct such as TxB4 (residues 767-2366) could be used. For purification of antibodies to Toxin A, a construct such as TxA4 (residues 770-2710) could be used. For affinity purification of antibodies which bind toxin B, serum which contains antibodies to Toxin B is diluted 1:1 with a suitable buffer (e.g. 20 mM HEPES pH 7.4 buffer containing 0.5M NaCl) and the mixture applied to column containing immobilised TxB4 packed in a suitable column (2-6 ml mixture per ml of gel). After the unbound fraction (which contains serum albumin and non-specific IgG) is washed off with at least 10 column volumes of 20 mM HEPES pH 7.4 buffer containing 0.5M NaCl buffer, the bound fraction is eluted from the column with 5 column volumes of elution buffer (e.g. 100mM glycine buffer, pH 2.5). The eluted fractions containing the IgG are then immediately neutralised to approximately pH 7.0 with of 1M Tris-HCl pH 8.0. These fractions, which contain the IgG which binds Toxin B, are then dialysed against 50mM HEPES pH 7.4 containing 0.15m NaCl and stored frozen until required

Affinity purified IgG fractions which bind and neutralises either Toxin A or B may be used as therapeutic agents to either treatment of prevent CDI. They may also be used in assay systems such as enzyme-linked immunosorbant assay (ELISA) for the detection of Toxins A

or B. In such diagnostic systems, affinity purified antibodies may provide assays of higher sensitivity and with reduced background interference.

Figures and Tables

Table 1 - Toxin A Receptor-Binding Repeat Modules

Toxin A Receptor-Binding Modules	**Amino acid Sequence** (Long Repeat regions shown in bold)
Module 1 LR = SEQ ID NO: 60	**GVFKGPDGFEYFAPANTQNNNIEGQAIVYQS**
Module 1 (residues 1851-2007 = SEQ ID NO: 61)	VTGWQTINGKKYYFDINTGAALTSYKIINGKHFYFNNDGVMQL**GVFKGPDGFEYFAPANTQNNNIEGQAIVYQS**KFLTLNGK YFDNNSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFN
Module 2 LR = SEQ ID NO: 62	**GVFSTSNGFEYFAPANTYNNNIEGQAIVYQS**
Module 2 (residues 2008-2141 = SEQ ID NO: 63)	GYKTIDGKHFYFDSDCVVKI**GVFSTSNGFEYFAPANTYNNNIEGQAIVYQS**KFLTLNGKKYYFDNNSKAVTGLQTIDSKKYY TNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIAST
Module 3 LR = SEQ ID NO: 64)	**GVFKGPNGFEYFAPANTDANNIEGQAILYQN**
Module 3 (residues 2142-2253 = SEQ ID NO: 65)	GYTIINGKHFYFNTDGIMQI**GVFKGPNGFEYFAPANTDANNIEGQAILYQN**EFLTLNGKKYYFGSDSKAVTGWRIINNKKYYF NNAIAAIHLCTINNDKYYFSYDGILQN
Module 4 LR = SEQ ID NO: 66)	**GVFKGPNGFEYFAPANTHNNNIEGQAIVYQN**
Module 4 (residues 2254-2389 = SEQ ID NO: 67)	GYITIERNNFYFDANNESKMVT**GVFKGPNGFEYFAPANTHNNNIEGQAIVYQN**KFLTLNGKKYYFDNDSKAVTGWQTIDGK YFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIAST
Module 5 LR = SEQ ID NO: 68)	**GVFKGPNGFEYFAPANTDANNIEGQAILYQN**
Module 5 (residues 2390-2502 = SEQ ID NO: 69)	GYTSINGKHFYFNTDGIMQI**GVFKGPNGFEYFAPANTDANNIEGQAILYQN**KFLTLNGKKYYFGSDSKAVTGLRTIDGKKYY TNTAVAVTGWQTINGKKYYFNTNTSIAST
Module 6 LR = SEQ ID NO: 70)	**GVFKGPDGFEYFAPANTDANNIEGQAIRYQN**
Module 6 (residues 2503-2594 = SEQ ID NO: 71)	GYTIISGKHFYFNTDGIMQI**GVFKGPDGFEYFAPANTDANNIEGQAIRYQN**RFLYLHDNIYYFGNNSKAATGWVTIDGNRYY PNTAMGAN
Module 7 LR = SEQ ID NO: 72)	**GVFKGSNGFEYFAPANTDANNIEGQAIRYQN**
Module 7 (residues 2595-2710 = SEQ ID NO: 73)	GYKTIDNKNFYFRNGLPQI**GVFKGSNGFEYFAPANTDANNIEGQAIRYQN**RFLHLLGKIYYFGNNSKAVTGWQTINGKVYYI PDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

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Table 2 - Toxin B Receptor-Binding Repeat Modules

Toxin B Receptor-Binding Modules	**Amino acid Sequence** (Long Repeat regions shown in bold)
Module 1 LR = SEQ ID NO: 74	**GVFSTEDGFKYFAPANTLDENLEGEAIDFT**
Module 1 (residues 1852-2007 = SEQ ID NO: 75)	DDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQT**GVFSTEDGFKYFAPANTLDENLEGEAIDFT**GKLIIDE NIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDS GVMKV
Module 2 LR = SEQ ID NO: 76	**GVFNTEDGFKYFAHHNEDLGNEEGEEISYS**
Module 2 (residues 2008-2139 = SEQ ID NO: 77)	GYTEIDGKHFYFAENGEMQI**GVFNTEDGFKYFAHHNEDLGNEEGEEISYS**GILNFNNKIYYFDDSFTAVVG WKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIES
Module 3 LR = SEQ ID NO: 78	**GVFDTSDGYKYFAPANTVNDNIYGQAVEYS**
Module 3 (residues 2140-2273 = SEQ ID NO: 79)	GVQNIDDNYFYIDDNGIVQI**GVFDTSDGYKYFAPANTVNDNIYGQAVEYS**GLVRVGEDVYYFGETYTIETG WIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQF
Module 4 LR = SEQ ID NO: 80	**GVFNTPDGFKYFAHQNTLDENFEGESINYT**
Module 4 (residues 2274-2366 = SEQ ID NO: 81)	GYINIEDKMFYFGEDGVMQI**GVFNTPDGFKYFAHQNTLDENFEGESINYT**GWLDLDEKRYYFTDEYIAATG SVIIDGEEYYFDPDTAQLVISE

Table 3 - Neutralisation titres obtained by immunisation of sheep with recombinant Toxin B-derived antigens (6 weeks time point; 2 doses of 100µg each)

Toxin B- derived Antigen (amino acid sequence)	Neutralisation titre against Toxin B (0.5ng/ml)
Recombinant Toxin B (1756-2366)	<10
Recombinant Toxin B (1145-2366)	960
Recombinant Toxin B (767-2366)	2,560
Recombinant Toxin B (543-2366)	1,280

Table 4 - Neutralisation titres obtained by immunisation of sheep with recombinant Toxin B-derived antigens (18 weeks time point; 5 doses of 100µg each)

Toxin B- derived Antigen (amino acid sequence)	Neutralisation titre against Toxin B (0.5ng/ml)
Recombinant Toxin B (1756-2366)	80
Recombinant Toxin B (1145-2366)	5,120
Recombinant Toxin B (767-2366)	10,250
Recombinant Toxin B (543-2366)	5,120

Table 5 - Neutralisation titres obtained by immunisation of sheep with a recombinant Toxin B-derived antigen (TxB4; residues 767-2366) of the invention

Antigen	No of Doses	Immunisation period (weeks)	Neutralisation titre against Toxin B (0.5ng/ml)
Recombinant Toxin B (residues 767-2366) at 100µg/dose	2	6	2,560
	3	10	2,560
	4	14	10,250
	5	18	10,250
	6	22	20,480

Table 6 - Neutralisation titres obtained by immunisation of sheep with a recombinant Toxin B-derived antigen (TxB2, 1756-2366) representing the repeat regions

Antigen	No of Doses	Immunisation period (weeks)	Neutralisation titre against Toxin B (0.5ng/ml)
Recombinant Toxin B (residues 767-2366) at 100µg/dose	2	6	<10
	3	10	10
	4	14	10
	5	18	80

Table 7 - Neutralisation titres obtained by immunisation of sheep with recombinant Toxin A-derived antigens (10 weeks time point)

Toxin A- derived Antigen (amino acid sequence)	No of Doses (100µg)	Neutralisation titre against Toxin A (50ng/ml)
Recombinant Toxin A (1850-2710)	3	640
Recombinant Toxin A (770-2710)	2	7,680
Recombinant Toxin A(770-2389)	3	10,240

Table 8 - Neutralisation titres obtained by immunisation of sheep with recombinant Toxin A-derived antigens (18 weeks time point)

Toxin A- derived Antigen (amino acid sequence)	No of Doses (100µg)	Neutralisation titre against Toxin A (50ng/ml)
Recombinant Toxin A (1850-2710)	5	1,280
Recombinant Toxin A (770-2710)	4	15,360

Table 9 - Neutralisation titres obtained by immunisation of sheep with a recombinant Toxin A-derived antigen (TxA4; residues 770-2710) of the invention

Antigen	No of Doses	Immunisation period (weeks)	Neutralisation titre against Toxin A (50ng/ml)
Recombinant Toxin A (residues 770-2710) at 100µg/dose	2	10	7,680
	3	14	10,240
	4	18	15, 360

Table 10 - Neutralisation titres obtained by immunisation of sheep with a recombinant Toxin A-derived antigen (TxA2; residues 1850-2710) representing the repeat region only

Antigen	No of Doses	Immunisation period (weeks)	Neutralisation titre against Toxin A (50ng/ml)
Recombinant Toxin A (residues 1850-2710) at 100µg/dose	2	6	320
	3	10	630
	4	14	1,280
	5	18	1,280

SED ID NOs

SEQ ID NO: 1 - *Clostridium difficile* Toxin A (Toxinotype 0)

MSLISKEELIKLAYSIRPRENEYKTILTNLDEYNKLTTNNNENKYLQLKKLNESIDVFMNKYKTSSRNRA
LSNLKKDILKEVILIKNSNTSPVEKNLHFVWIGGEVSDIALEYIKQWADINAEYNIKLWYDSEAFLVNTLK
KAIVESSTTEALQLLEEEIQNPQFDNMKFYKKRMEFIYDRQKRFINYYKSQINKPTVPTIDDIIKSHLVSE
YNRDETVLESYRTNSLRKINSNHGIDIRANSLFTEQELLNIYSQELLNRGNLAAASDIVRLLALKNFGGV
YLDVDMLPGIHSDLFKTISRPSSIGLDRWEMIKLEAIMKYKKYINNYTSENFDKLDQQLKDNFKLIIESKS
EKSEIFSKLENLNVSDLEIKIAFALGSVINQALISKQGSYLTNLVIEQVKNRYQFLNQHLNPAIESDNNFT
DTTKIFHDSLFNSATAENSMFLTKIAPYLQVGFMPEARSTISLSGPGAYASAYYDFINLQENTIEKTLKA
SDLIEFKFPENNLSQLTEQEINSLWSFDQASAKYQFEKYVRDYTGGSLSEDNGVDFNKNTALDKNYLL
NNKIPSNNVEEAGSKNYVHYIIQLQGDDISYEATCNLFSKNPKNSIIIQRNMNESAKSYFLSDDGESILE
LNKYRIPERLKNKEKVKVTFIGHGKDEFNTSEFARLSVDSLSNEISSFLDTIKLDISPKNVEVNLLGCNM
FSYDFNVEETYPGKLLLSIMDKITSTLPDVNKNSITIGANQYEVRINSEGRKELLAHSGKWINKEEAIMS
DLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEP
VKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETE
KEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVK
VQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKEL
EAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSESKK
YGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYS
AIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDY
AITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWI
FNIDNEVRESIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLV
AKSYSLLLSGDKNYLISNLSNTIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILE
FYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSS
YLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFG
EWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNY
YSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFN
KMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIE
FNLVTGWQTINGKKYYFDINTGAALTSYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNI
EGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIIS
KGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQ
AIVYQSKFLTLNGKKYYFDNNSKAVTGLQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAAT
GWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAIL
YQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIE
RNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVT
GWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSI
NGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGL
RTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGF
EYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTI
DNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGW
QTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID NO: 2 - *C. difficile* Toxin B (Toxinotype 0)

MSLVNRKQLEKMANVRFRTQEDEYVAILDALEEYHNMSENTVVEKYLKLKDINSLTDIYIDTYKKSGRN
KALKKFKEYLVTEVLELKNNNLTPVEKNLHFVWIGGQINDTAINYINQWKDVNSDYNVNVFYDSNAFLI
NTLKKTVVESAINDTLESFRENLNDPRFDYNKFFRKRMEIIYDKQKNFINYYKAQREENPELIIDDIVKTY
LSNEYSKEIDELNTYIEESLNKITQNSGNDVRNFEEFKNGESFNLYEQELVERWNLAAASDILRISALKE
IGGMYLDVDMLPGIQPDLFESIEKPSSVTVDFWEMTKLEAIMKYKEYIPEYTSEHFDMLDEEVQSSFE
SVLASKSDKSEIFSSLGDMEASPLEVKIAFNSKGIINQGLISVKDSYCSNLIVKQIENRYKILNNSLNPAIS
EDNDFNTTTNTFIDSIMAEANADNGRFMMELGKYLRVGFFPDVKTTINLSGPEAYAAAYQDLLMFKEG
SMNIHLIEADLRNFEISKTNISQSTEQEMASLWSFDDARAKAQFEEYKRNYFEGSLGEDDNLDFSQNI
VVDKEYLLEKISSLARSSERGYIHYIVQLQGDKISYEAACNLFAKTPYDSVLFQKNIEDSEIAYYYNPGD
GEIQEIDKYKIPSIISDRPKIKLTFIGHGKDEFNTDIFAGFDVDSLSTEIEAAIDLAKEDISPKSIEINLLGCN
MFSYSINVEETYPGKLLLKVKDKISELMPSISQDSIIVSANQYEVRINSEGRRELLDHSGEWINKEESIIK
DISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEERIEE
AKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEK
TIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKV
QVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEI

EAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVET
EGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSI
YDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFA
FIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSES
DVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAI
IEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVS
ELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHL
DESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDEN
DNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYT
DEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNV
FKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYIND
SLYYFKPPVNNLITGFVTVGD
DKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLII
DENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKH
YFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNN
KIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFS
DSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYF
GETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGE
MQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTD
EYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID NO:3 - *C. difficile* Toxin A (Toxinotype 3)

MSLISKEELIKLAYSIRPRENEYKTILTNLDEYNKLTTNNNENKYLQLKKLNESIDVFMNKYKNSSRNRA
LSNLKKDILKEVILIKNSNTSPVEKNLHFVWIGGEVSDIALEYIKQWADINAEYNIKLWYDSEAFLVNTLK
KAIVESSTTEALQLLEEEIQNPQFDNMKFYKKRMEFIYDRQKRFINYYKSQINKPTVPTIDDIIKSHLVSE
YNRDETLLESYRTNSLRKINSNHGIDIRANSLFTEQELLNIYSQELLNRGNLAAASDIVRLLALKNFGGV
YLDVDMLPGIHSDLFKTIPRPSSIGLDRWEMIKLEAIMKYKKYINNYTSENFDKLDQQLKDNFKLIIESKS
EKSEIFSKLENLNVSDLEIKIAFALGSVINQALISKQGSYLTNLVIEQVKNRYQFLNQHLNPAIESDNNFT
DTTKIFHDSLFNSATAENSMFLTKIAPYLQVGFMPEARSTISLSGPGAYASAYYDFINLQENTIEKTLKA
SDLIEFKFPENNLSQLTEQEINSLWSFDQASAKYQFEKYVRDYTGGSLSEDNGVDFNKNTALDKNYLL
NNKIPSNNVEEAGSKNYVHYIIQLQGDDISYEATCNLFSKNPKNSIIIQRNMNESAKSYFLSDDGESILE
LNKYRIPERLKNKEKVKVTFIGHGKDEFNTSEFARLSVDSLSNEISSFLDTIKLDISPKNVEVNLLGCNM
FSYDFNVEETYPGKLLLSIMDKITSTLPDVNKDSITIGANQYEVRINSEGRKELLAHSGKWINKEEAIMS
DLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEP
VKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETE
KEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVK
VQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKEL
EAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSESKE
YGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPYISSHIPSLSVYS
AIGIKTENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENNGTKLLDSIRDLYPGKFYWRFYAFFDY
AITTLKPVYEDTNTKIKLDKDTRNFIMPTITTDEIRNKLSYSFDGAGGTYSLLLSSYPISMNINLSKDDLWI
FNIDNEVRESIENGTIKKGNLIEDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLV
AKSYSLLLSGDKNYLISNLSNTIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILE
FYNGSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSS
YLDFVKNSDGHHNTSNFMNLFLNNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFG
EWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNY
YSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFN
KMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIE
SNLVTGWQTINGKKYYFDINTGAASTSYKIINGKHFYFNNNGVMQLGVFKGPDGFEYFAPANTQNNNI
EGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIIS
KGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSGSNGFEYFAPANTYNNNIEGQ
AIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAAT
GWQTIDGKKYYFNTNTSIASTGYTIINGKYFYFNTDGIMQIGVFKVPNGFEYFAPANTHNNNIEGQAILY
QNKFLTLNGKKYYFGSDSKAITGWQTIDGKKYYFNPNNAIAATHLCTINNDKYYFSYDGILQNGYITIER
NNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTG
WQTIDSKKYYFNLNTAVAVTGWQTIDGEKYYFNLNTAEAATGWQTIDGKRYYFNTNTYIASTGYTIING
KHFYFNTDGIMQIGVFKGPDGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTI
DGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTYIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYF
APANTDANNIEGQAIRYQNRFLYLHDNIYYFGNDSKAATGWATIDGNRYYFEPNTAMGANGYKTIDNK

NFYFRNGLPQIGVFKGPNGFEYFAPANTDANNIDGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTIN
SKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID NO: 4 - *C. difficile* Toxin B (Toxinotype 3)

MSLVNRKQLEKMANVRFRVQEDEYVAILDALEEYHNMSENTVVEKYLKLKDINSLTDIYIDTYKKSGR
NKALKKFKEYLVTEVLELKNNNLTPVEKNLHFVWIGGQINDTAINYINQWKDVNSDYNVNVFYDSNAF
LINTLKKTIVESATNDTLESFRENLNDPRFDYNKFYRKRMEIIYDKQKNFINYYKTQREENPDLIIDDIVKI
YLSNEYSKDIDELNSYIEESLNKVTENSGNDVRNFEEFKGGESFKLYEQELVERWNLAAASDILRISAL
KEVGGVYLDVDMLPGIQPDLFESIEKPSSVTVDFWEMVKLEAIMKYKEYIPGYTSEHFDMLDEEVQSS
FESVLASKSDKSEIFSSLGDMEASPLEVKIAFNSKGIINQGLISVKDSYCSNLIVKQIENRYKILNNSLNP
AISEDNDFNTTTNAFIDSIMAEANADNGRFMMELGKYLRVGFFPDVKTTINLSGPEAYAAAYQDLLMF
KEGSMNIHLIEADLRNFEISKTNISQSTEQEMASLWSFDDARAKAQFEEYKKNYFEGSLGEDDNLDFS
QNTVVDKEYLLEKISSLARSSERGYIHYIVQLQGDKISYEAACNLFAKTPYDSVLFQKNIEDSEIAYYYN
PGDGEIQEIDKYKIPSIISDRPKIKLTFIGHGKDEFNTDIFAGLDVDSLSTEIETAIDLAKEDISPKSIEINLL
GCNMFSYSVNVEETYPGKLLLRVKDKVSELMPSISQDSIIVSANQYEVRINSEGRRELLDHSGEWINK
EESIIKDISSKEYISFNPKENKIIVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLAECEINVISNIDTQVV
EGRIEEAKSLTSDSINYIKNEFKLIESISDALYDLKQQNELEESHFISFEDILETDEGFSIRFIDKETGESIF
VETEKAIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDATHEVNTLNAAFFIQSLIEYNSSKESLSNLS
VAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPVIATIIDGVSLGAAIKELSETSD
PLLRQEIEAKIGIMAVNLTAATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELILRDKATKVVDYFSHI
SLAESEGAFTSLDDKIMMPQDDLVISEIDFNNNSITLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYR
EPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFY
WRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPVITTEYIREKLSYSFYGSGGTYALSLSQYNMNI
NIELNENDTWVIDVDNVVRDVTIESDKIKKGDLIENILSKLSIEDNKIILDNHEINFSGTLNGGNGFVSLTF
SILEGINAVIEVDLLSKSYKVLISGELKTLMANSNSVQQKIDYIGLNSELQKNIPYSFMDDKGKENGFINC
STKEGLFVSELSDVVLISKVYMDNSKPLFGYCSNDLKDVKVITKDDVIILTGYYLKDDIKISLSFTIQDEN
TIKLNGVYLDENGVAEILKFMNKKGSTNTSDSLMSFLESMNIKSIFINSLQSNTKLILDTNFIISGTTSIGQ
FEFICDKDNNIQPYFIKFNTLETKYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNK
VIISPNIYTDEINITPIYEANNTYPEVIVLDTNYISEKININDLSIRYVWSNDGSDFILMSTDEENKVSQV
KIRFTNVFKGNTISDKISFNFSDKQDVSINKVISTFTPSYYVEGLLNYDLGLISLYNEKFYINNFGMMVS
GLVYINDSLYYFKPPIKNLITGFTTIGDDKYYFNPDNGGAASVGETIIDGKNYYFSQNGVLQTGVFSTE
DGFKYFAPADTLDENLEGEAIDFTGKLTIDENVYYFGDNYRAAIEWQTLDDEVYYFSTDTGRAFKGLN
QIGDDKFYFNSDGIMQKGFVNINDKTFYFDDSGVMKSGYTEIDGKYFYFAENGEMQIGVFNTADGFK
YFAHHDEDLGNEEGEALSYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGISIIND
GKYYFNDSGIMQIGFVTINNEVFYFSDSGIVESGMQNIDDNYFYIDENGLVQIGVFDTSDGYKYFAPAN
TVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFDPETKKAYKGINVIDDIKYY
FDENGIMRTGLITFEDNHYYFNEDGIMQYGYLNIEDKTFYFSEDGIMQIGVFNTPDGFKYFAHQNTLDE
NFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 5 - Toxin A fragment - TxA3 (Toxinotype 0) (Residues 1131-2710)

ESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSL
SIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYA
FFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKD
DLWIFNIDNEVRESIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLI
EINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDS
KNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNES
VYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNI
DIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLINI
NTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNT
QSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYF
DPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQN
NNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDT
AIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIE
GQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAE
AATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQ
AILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYI
TIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSK
AVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTG
YTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKA
VTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKG

PDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGAN
GYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKA
VTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 6 - Toxin A fragment - TxA3 (Toxinotype 3) (Residues 1131-2710)
ESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSL
SIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYA
FFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKD
DLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLII
EINLVAKSYSLLLSGDKNYLISNLSNTIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDS
KNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNES
VYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNI
DIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLINI
NTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNT
QSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYF
DPIEFNLVTGWQTINGKKYYFDINTGAALTSYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQ
NNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPD
TAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNI
EGQAIVYQSKFLTLNGKKYYFDNNSKAVTGLQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAE
AATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQ
AILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYI
TIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSK
AVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTG
YTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNKFLTLNGKKYYFGSDSKA
VTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKG
PDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGAN
GYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKA
VTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 7 - Toxin A fragment - TxA4 (Toxinotype 0) (Residues 770-2710)
IMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKL
EPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVE
TEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTS
VKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLK
KELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSES
KKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSI
YSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFF
DYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDL
WIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEIN
LVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNI
LEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVY
SSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIY
FGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININT
NYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQS
FNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDP
IEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNN
NIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTA
IISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEG
QAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEA
ATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQA
ILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITI
ERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAV
TGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTS
INGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTG
LRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDG
FEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYK
TIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTG
WQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 8 - Toxin A fragment - TxA4 (Toxinotype 3) (Residues 770-2710)

IMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKL
EPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVE
TEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTS
VKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLK
KELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSES
KEYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPYISSHIPSLSV
YSAIGIKTENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENNGTKLLDSIRDLYPGKFYWRFYAFF
DYAITTLKPVYEDTNTKIKLDKDTRNFIMPTITTDEIRNKLSYSFDGAGGTYSLLLSSYPISMNINLSKDD
LWIFNIDNEVREISIENGTIKKGNLIEDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEI
NLVAKSYSLLLSGDKNYLISNLSNTIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSK
NILEFYNGSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESV
YSSYLDFVKNSDGHHNTSNFMNLFLNNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDI
YFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININ
TNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQ
SFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFD
PIESNLVTGWQTINGKKYYFDINTGAASTSYKIINGKHFYFNNNGVMQLGVFKGPDGFEYFAPANTQN
NNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDT
AIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSGSNGFEYFAPANTYNNNIE
GQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAE
AATGWQTIDGKKYYFNTNTSIASTGYTIINGKYFYFNTDGIMQIGVFKVPNGFEYFAPANTHNNNIEGQ
AILYQNKFLTLNGKKYYFGSDSKAITGWQTIDGKKYYFNPNNAIAATHLCTINNDKYYFSYDGILQNGYI
TIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSK
AVTGWQTIDSKKYYFNLNTAVAVTGWQTIDGEKYYFNLNTAEAATGWQTIDGKRYYFNTNTYIASTG
YTIINGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAV
TGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTYIASTGYTIISGKHFYFNTDGIMQIGVFKGP
DGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNDSKAATGWATIDGNRYYFEPNTAMGANG
YKTIDNKNFYFRNGLPQIGVFKGPNGFEYFAPANTDANNIDGQAIRYQNRFLHLLGKIYYFGNNSKAVT
GWQTINSKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 9 - Toxin B fragment - TxB3(-h) (Toxinotype 0) (Residues 1145-2366)
MPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEEL
DLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPR
YEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVR
DVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKL
LISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVY
MDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKF
MNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNT
LETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETN
NTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLS
FNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNN
LITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEG
EAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKG
FVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEIS
YSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTI
NDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVR
VGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNN
YYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLD
EKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 10 - Toxin B fragment - TxB3(-h) (Toxinotype 3) (Residues 1145-2366)
MPQDDLVISEIDFNNNSITLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEEL
DLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPR
YEDTNIRINLDSNTRSFIVPVITTEYIREKLSYSFYGSGGTYALSLSQYNMNINIELNENDTWVIDVDNVV
RDVTIESDKIKKGDLIENILSKLSIEDNKIILDNHEINFSGTLNGGNGFVSLTFSILEGINAVIEVDLLSKSY
KVLISGELKTLMANSNSVQQKIDYIGLNSELQKNIPYSFMDDKGKENGFINCSTKEGLFVSELSDVVLIS
KVYMDNSKPLFGYCSNDLKDVKVITKDDVIILTGYYLKDDIKISLSFTIQDENTIKLNGVYLDENGVAEIL
KFMNKKGSTNTSDSLMSFLESMNIKSIFINSLQSNTKLILDTNFIISGTTSIGQFEFICDKDNNIQPYFIKF
NTLETKYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVIISPNIYTDEINITPIYEA
NNTYPEVIVLDTNYISEKININDLSIRYVWSNDGSDFILMSTDEENKVSQVKIRFTNVFKGNTISDKISF
NFSDKQDVSINKVISTFTPSYYVEGLLNYDLGLISLYNEKFYINNFGMMVSGLVYINDSLYYFKPPIKNLI

TGFTTIGDDKYYFNPDNGGAASVGETIIDGKNYYFSQNGVLQTGVFSTEDGFKYFAPADTLDENLEGE
AIDFTGKLTIDENVYYFGDNYRAAIEWQTLDDEVYYFSTDTGRAFKGLNQIGDDKFYFNSDGIMQKGF
VNINDKTFYFDDSGVMKSGYTEIDGKYFYFAENGEMQIGVFNTADGFKYFAHHDEDLGNEEGEALSY
SGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGISIINDGKYYFNDSGIMQIGFVTINN
EVFYFSDSGIVESGMQNIDDNYFYIDENGLVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRV
GEDVYYFGETYTIETGWIYDMENESDKYYFDPETKKAYKGINVIDDIKYYFDENGIMRTGLITFEDNHY
YFNEDGIMQYGYLNIEDKTFYFSEDGIMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEK
RYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 11 - Toxin B fragment - TxB3 (Toxinotype 0) (Residues 957-2366)
NTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSE
GLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGI
PSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRM
EGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGL
RSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKL
SYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIIL
NSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSEL
QKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNV
NILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFV
NFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDS
GDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIR
YVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGL
IGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGE
TIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVE
WKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEID
GKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWK
DLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYF
YIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMEN
ESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFG
EDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYY
FDPDTAQLVISE

SEQ ID 12 - Toxin B fragment - TxB3 (Toxinotype 3) (Residues 957-2366)
NTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSE
GLPVIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTAATTAIITSSLGIASGFSILLVPLAGISA
GIPSLVNNELILRDKATKVVDYFSHISLAESEGAFTSLDDKIMMPQDDLVISEIDFNNNSITLGKCEIWR
MEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTP
GLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPVITTEYIR
EKLSYSFYGSGGTYALSLSQYNMNINIELNENDTWVIDVDNVVRDVTIESDKIKKGDLIENILSKLSIEDN
KIILDNHEINFSGTLNGGNGFVSLTFSILEGINAVIEVDLLSKSYKVLISGELKTLMANSNSVQQKIDYIGL
NSELQKNIPYSFMDDKGKENGFINCSTKEGLFVSELSDVVLISKVYMDNSKPLFGYCSNDLKDVKVITK
DDVIILTGYYLKDDIKISLSFTIQDENTIKLNGVYLDENGVAEILKFMNKKGSTNTSDSLMSFLESMNIKSI
FINSLQSNTKLILDTNFIISGTTSIGQFEFICDKDNNIQPYFIKFNTLETKYTLYVGNRQNMIVEPNYDLDD
SGDISSTVINFSQKYLYGIDSCVNKVIISPNIYTDEINITPIYEANNTYPEVIVLDTNYISEKININDLSIRY
VWSNDGSDFILMSTDEENKVSQVKIRFTNVFKGNTISDKISFNFSDKQDVSINKVISTFTPSYYVEGLL
NYDLGLISLYNEKFYINNFGMMVSGLVYINDSLYYFKPPIKNLITGFTTIGDDKYYFNPDNGGAASVGET
IIDGKNYYFSQNGVLQTGVFSTEDGFKYFAPADTLDENLEGEAIDFTGKLTIDENVYYFGDNYRAAIEW
QTLDDEVYYFSTDTGRAFKGLNQIGDDKFYFNSDGIMQKGFVNINDKTFYFDDSGVMKSGYTEIDGK
YFYFAENGEMQIGVFNTADGFKYFAHHDEDLGNEEGEALSYSGILNFNNKIYYFDDSFTAVVGWKDL
EDGSKYYFDEDTAEAYIGISIINDGKYYFNDSGIMQIGFVTINNEVFYFSDSGIVESGMQNIDDNYFYID
ENGLVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENES
DKYYFDPETKKAYKGINVIDDIKYYFDENGIMRTGLITFEDNHYYFNEDGIMQYGYLNIEDKTFYFSED
GIMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDP
DTAQLVISE

SEQ ID 13 - Toxin B fragment - TxB 4 (Toxinotype 0) (Residues 767-2366)
SIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEE
RIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVE
TEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVA
MKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLL

RQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSL
VETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREP
HLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWR
YFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIEL
SESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEG
INAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGL
FVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNS
VHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICD
ENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPN
IYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFV
NVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYI
NDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKY
FAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDY
KYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAH
HNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQY
YFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVN
DNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDE
KGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDEN
FEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 14 - Toxin B fragment - TxB 4 (Toxinotype 3) (Residues 767-2366)

SIIKDISSKEYISFNPKENKIIVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLAECEINVISNIDTQVVEG
RIEEAKSLTSDSINYIKNEFKLIESISDALYDLKQQNELEESHFISFEDILETDEGFSIRFIDKETGESIFVE
TEKAIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDATHEVNTLNAAFFIQSLIEYNSSKESLSNLSVA
MKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPVIATIIDGVSLGAAIKELSETSDPLL
RQEIEAKIGIMAVNLTAATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELILRDKATKVVDYFSHISLA
ESEGAFTSLDDKIMMPQDDLVISEIDFNNNSITLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPH
LSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRY
FAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPVITTEYIREKLSYSFYGSGGTYALSLSQYNMNINIEL
NENDTWVIDVDNVVRDVTIESDKIKKGDLIENILSKLSIEDNKIILDNHEINFSGTLNGGNGFVSLTFSILE
GINAVIEVDLLSKSYKVLISGELKTLMANSNSVQQKIDYIGLNSELQKNIPYSFMDDKGKENGFINCSTK
EGLFVSELSDVVLISKVYMDNSKPLFGYCSNDLKDVKVITKDDVIILTGYYLKDDIKISLSFTIQDENTIKL
NGVYLDENGVAEILKFMNKKGSTNTSDSLMSFLESMNIKSIFINSLQSNTKLILDTNFIISGTTSIGQFEFI
CDKDNNIQPYFIKFNTLETKYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVIIS
PNIYTDEINITPIYEANNTYPEVIVLDTNYISEKININDLSIRYVWSNDGSDFILMSTDEENKVSQVKIRF
TNVFKGNTISDKISFNFSDKQDVSINKVISTFTPSYYVEGLLNYDLGLISLYNEKFYINNFGMMVSGLVYI
NDSLYYFKPPIKNLITGFTTIGDDKYYFNPDNGGAASVGETIIDGKNYYFSQNGVLQTGVFSTEDGFKY
FAPADTLDENLEGEAIDFTGKLTIDENVYYFGDNYRAAIEWQTLDDEVYYFSTDTGRAFKGLNQIGDD
KFYFNSDGIMQKGFVNINDKTFYFDDSGVMKSGYTEIDGKYFYFAENGEMQIGVFNTADGFKYFAHH
DEDLGNEEGEALSYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGISIINDGKYYF
NDSGIMQIGFVTINNEVFYFSDSGIVESGMQNIDDNYFYIDENGLVQIGVFDTSDGYKYFAPANTVNDN
IYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFDPETKKAYKGINVIDDIKYYFDENG
IMRTGLITFEDNHYYFNEDGIMQYGYLNIEDKTFYFSEDGIMQIGVFNTPDGFKYFAHQNTLDENFEGE
SINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 15 - Toxin B fragment - Toxin B-A hybrid (toxinotype 0)

NTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSE
GLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGI
PSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRM
EGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGL
RSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKL
SYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIIL
NSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSEL
QKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNV
NILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFV
NFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDS
GDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIR
YVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGL
IGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLVTGWQTINGKKYYFDINTGAALISYK
IINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVT

GWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTID
GKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGW
QTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGK
HFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIIN
NKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYF
APANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDG
KKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFA
PANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKK
YYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHD
NIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAP
ANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVI
YFFGVDGVKAPGIYG

SEQ ID 16 - Toxin B fragment - Toxin A-B hybrid (toxinotype 0)

IMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKL
EPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVE
TEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTS
VKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLK
KELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSES
KKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSI
YSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFF
DYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDL
WIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEIN
LVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNI
LEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVY
SSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIY
FGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININT
NYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQS
FNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDP
IEFNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDEN
LEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVM
QKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEG
EEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVG
FVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSG
LVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFEN
NNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLD
LDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 17 - Toxin B fragment - Toxin A-B hybrid (toxinotype 0 and 3)

IMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKL
EPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVE
TEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTS
VKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLK
KELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSES
KKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSI
YSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFF
DYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDL
WIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEIN
LVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNI
LEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVY
SSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIY
FGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININT
NYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQS
FNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDP
IEFNLITGFTTIGDDKYYFNPDNGGAASVGETIIDGKNYYFSQNGVLQTGVFSTEDGFKYFAPADTLDE
NLEGEAIDFTGKLTIDENVYYFGDNYRAAIEWQTLDDEVYYFSTDTGRAFKGLNQIGDDKFYFNSDGI
MQKGFVNINDKTFYFDDSGVMKSGYTEIDGKYFYFAENGEMQIGVFNTADGFKYFAHHDEDLGNEE
GEALSYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGISIINDGKYYFNDSGIMQIG
FVTINNEVFYFSDSGIVESGMQNIDDNYFYIDENGLVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYS
GLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFDPETKKAYKGINVIDDIKYYFDENGIMRTGLITF

EDNHYYFNEDGIMQYGYLNIEDKTFYFSEDGIMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWL
DLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 18 - Toxin A-derived recombinant antigen His-NusA-[thrombin site]-TxA4-His

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALVPRGSVTSLYKKAGSAAAPFTMIMSDLSSKEYIFFDSIDN
KLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEF
NLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEIS
TIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTI
YDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSI
AATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPID
DLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIM
MLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKI
KLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGT
IKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYL
ISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIA
EDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTS
NFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGN
GRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNT
FHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYI
MSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYY
FDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGK
KYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDT
DTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYY
FDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNT
AIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGS
DSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTG
VFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTA
EAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGV
FKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVA
VTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQA
IRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVF
KGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMA
AAGGLFEIDGVIYFFGVDGVKAPGIYGGGSGGSLVPRGSGGSHHHHHH

SEQ ID 19 - Toxin A-derived recombinant antigen His-NusA-[thrombin site]-TxA4

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALVPRGSVTSLYKKAGSAAAPFTMIMSDLSSKEYIFFDSIDN
KLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEF
NLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEIS
TIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTI
YDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSI
AATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPID
DLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIM
MLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKI
KLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGT
IKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYL
ISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIA

EDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTS
NFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGN
GRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNT
FHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYI
MSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYY
FDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGK
KYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDT
DTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYY
FDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNT
AIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGS
DSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTG
VFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTA
EAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGV
FKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVA
VTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQA
IRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVF
KGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMA
AAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 20 - Toxin A-derived recombinant antigen - His-Thioredoxin-[thrombin site]-TxA4
HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSA
AAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGD
YIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNG
ESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVL
NDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDE
HDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYF
NHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISS
HIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYW
RFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNIN
LSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDD
KISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHY
KKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGL
YLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICD
NNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLY
TSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGI
LSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININN
SLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAP
ANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYY
FNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTY
NNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNT
NTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANN
IEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQ
NGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDN
DSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIA
STGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSD
SKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVF
KGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMG
ANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNS
KAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 21 - Toxin A-derived recombinant antigen - His-Thioredoxin-[thrombin site]-TxA3
HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSA
AAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSI
SSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKF
YWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPIST
NINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCEL
DDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSII
HYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVN

GLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFI
CDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAP
DLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIR
IKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLINI
NNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYF
APANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNK
YYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPAN
TYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYF
NTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDA
NNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGI
LQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYF
DNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTF
IASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGS
DSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIG
VFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTA
MGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGN
NSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 22 - Toxin A-derived recombinant antigen - His-NusA-[thrombin site]-TxA3

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALVPRGSVTSLYKKAGSAAAPFTMESKKYGPLKTEDDKILV
PIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKK
IMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNI
KIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIEN
GTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDK
NYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSK
DFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGH
HNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTI
FSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVL
NPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKL
SLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTIN
GKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFL
TLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSR
YYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLN
GKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKY
YFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGK
KYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNE
SKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYY
FNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGI
MQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNT
NTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANN
IEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLP
QIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPD
TAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 23 - Toxin B-derived recombinant antigen - His-NusA-[thrombin site]-TxB4-His

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALVPRGSVTSLYKKAGSAAAPFTMSIIKDISSKEYISFNPKE

NKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKD
EFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEIS
KIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNT
ITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTA
TTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMP
QDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLS
KDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYED
TNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVT
IESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISG
ELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDD
SKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRK
GNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETN
YTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTY
PEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNF
SDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITG
FVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAID
FTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSI
NDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGI
LNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDK
VFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGE
DVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYF
NENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKR
YYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISEGHHHHHH

SEQ ID 24 - Toxin B-derived recombinant antigen - His-NusA-[thrombin site]-TxB4

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALVPRGSVTSLYKKAGSAAAPFTMSIIKDISSKEYISFNPKE
NKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKD
EFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEIS
KIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNT
ITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTA
TTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMP
QDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLS
KDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYED
TNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVT
IESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISG
ELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDD
SKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRK
GNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETN
YTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTY
PEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNF
SDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITG
FVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAID
FTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSI
NDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGI
LNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDK
VFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGE
DVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYF
NENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKR
YYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 25 - Toxin B-derived recombinant antigen - His-Thioredoxin-[thrombin site]-TxB4

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSA

AAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNID
TQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETG
ESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESL
SNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSE
TSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDY
FKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPS
ITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEG
EFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNM
GINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLT
FSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGS
TKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEK
TIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQ
FEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNK
VVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQ
VKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMV
SGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTE
DGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLN
QIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGF
KYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLIN
DGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPA
NTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKY
YFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNT
LDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 26 - Toxin B-derived recombinant antigen - His-NusA-[thrombin site]-TxB3

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALVPRGSVTSLYKKAGSAAGGSMPQDDLVISEIDFNNNSIV
LGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFA
WETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIV
PIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGIL
STLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQK
IDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKD
VKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLE
SMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVE
PNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKIN
VNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFT
PSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPIN
GGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDD
NYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVM
KVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSF
TAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGV
QNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETG
WIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIE
DKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVI
IDGEEYYFDPDTAQLVISE

SEQ ID 27 - Toxin B-derived recombinant antigen - His-Thioredoxin-[thrombin site]-TxB3 (-hyd)

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSA
AGGSMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQ
KEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITT
LKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVD
NVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSK
SYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLI

SKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAE
ILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIK
FNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVY
ETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLAN
KLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPP
VNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDEN
LEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVM
QKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEG
EEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVG
FVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSG
LVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFEN
NNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLD
LDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 28 - Toxin A-derived recombinant antigen - His-[linear spacer]-NusA-[thrombin site]-TxA4

HHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASNKEILAVVEAVSNEKALPRE
KIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDESLNLG
DYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLGNNAE
AVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARD
PGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPADVASIV
VDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTFTKYLD
IDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDNKPADD
LLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEASGALVP
RGSVTSLYKKAGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTK
FILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKN
NSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFF
IQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILD
GINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNE
LILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVT
GNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLL
DSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTY
SLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDN
KDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYF
GAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSIS
LVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLV
GKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGI
DRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLE
ESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYD
EDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLG
VFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAI
AAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFS
TSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAAT
GWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPN
GFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTI
NNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQN
KFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTI
DGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKF
LTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKH
FYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTID
GNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRF
LHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG.

SEQ ID 29 - Toxin A-derived recombinant antigen - His-[helical spacer]-NusA-[thrombin site]-TxA4

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASNKEILAVVEAVSNEKA
LPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDES
LNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLG
NNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKA
AARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPAD

VASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTF
TKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDN
KPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEAS
GALVPRGSVTSLYKKAGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASV
SPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISF
EDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNT
LNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPI
VSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPS
LVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGS
GHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLEND
GTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDG
AGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFS
GDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESN
NKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSI
DFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKY
FTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEP
LYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILV
RYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTY
YYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVM
QLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPN
NAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIG
VFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAE
AATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFK
GPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIH
LCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIV
YQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATG
WQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILY
QNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTII
SGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATG
WVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIR
YQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIY
G

SEQ ID 30 - Toxin A-derived recombinant antigen - His-NusA-[linear spacer]- [thrombin site]-TxA4

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALGGSGGSGGSGGSGGSGGSGGSGGSGGSLVPRGSGS
AAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIG
DYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSN
GESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDV
LNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDE
HDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYF
NHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISS
HIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYW
RFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNIN
LSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDD
KISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHY
KKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGL
YLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICD
NNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLY
TSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGI
LSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININN
SLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAP
ANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYY

FNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTY
NNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNT
NTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANN
IEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQ
NGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDN
DSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIA
STGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSD
SKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVF
KGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMG
ANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNS
KAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 31 - Toxin A-derived recombinant antigen - His-NusA-[helical spacer]-[thrombin site]-TxA4

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSLVPRG
SGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIE
SSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFIN
KSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSN
KDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKE
LLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSV
VNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSS
PSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPG
KFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPI
STNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTC
ELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQK
SIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKV
NGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVE
FICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIA
PDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKI
RIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLI
NINNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFE
YFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDN
NKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAP
ANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKK
YYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPAN
TDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSY
DGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKK
YYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNT
NTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYY
FGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIM
QIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPN
TAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYF
GNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 32 - Toxin A-derived recombinant antigen - His-[linear spacer]-NusA-[thrombin site]-TxA3

HHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASNKEILAVVEAVSNEKALPRE
KIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDESLNLG
DYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLGNNAE
AVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARD
PGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPADVASIV
VDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTFTKYLD
IDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDNKPADD

LLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEASGALVP
RGSVTSLYKKAGSAAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGH
TVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGT
RLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAG
GTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGD
IDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNK
YFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFS
ISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTL
VGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLY
GIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRY
LEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYY
DEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQL
GVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNN
AIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGV
FSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEA
ATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKG
PNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHL
CTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVY
QNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGW
QTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQ
NKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIIS
GKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGW
VTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQ
NRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 33 - Toxin A-derived recombinant antigen - His-[helical spacer]-NusA-[thrombin site]-TxA3

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASNKEILAVVEAVSNEKA
LPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDES
LNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLG
NNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKA
AARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPAD
VASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTF
TKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDN
KPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEAS
GALVPRGSVTSLYKKAGSAAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAME
GGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRS
LENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSY
SFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQ
TIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYT
DESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNT
DKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFV
IDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDF
SYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGS
DFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKII
DNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNN
DGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKY
YFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDC
VVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFN
TNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIM
QIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPN
NAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNI
EGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTA
EAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIE
GQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIA
STGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNS
KAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIE
GQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVK
APGIYG

SEQ ID 34 - Toxin A-derived recombinant antigen - His-NusA-[linear spacer]- [thrombin site]-TxA3

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALGGSGGSGGSGGSGGSGGSGGSGGSGGSLVPRGSGS
AAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSP
SISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGK
FYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPIS
TNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCE
LDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSI
IHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVN
GLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFI
CDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAP
DLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIR
IKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLINI
NNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYF
APANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNK
YYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPAN
TYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYF
NTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDA
NNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGI
LQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYF
DNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTF
IASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGS
DSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIG
VFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTA
MGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGN
NSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 35 - Toxin A-derived recombinant antigen - His-NusA-[helical spacer]-[thrombin site]-TxA3

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSLVPRG
SGSAAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFF
SSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLY
PGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSY
PISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLT
CELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQ
KSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVK
VNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYV
EFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLI
APDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQ
KIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKG
LININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGF
EYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVID
NNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYF
APANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDG
KKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAP
ANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYF

SYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNG
KKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYF
NTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKK
YYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDG
IMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFE
PNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIY
YFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 36 - Toxin A-derived recombinant antigen - His-[linear spacer]-Thioredoxin- [thrombin site]-TxA4

HHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASDKIIHLTDDSFDTDVLKADGA
ILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVG
ALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIP
GLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDEL
YELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVN
GNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLIS
NAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIG
AEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNN
NSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVF
WWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIM
PTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLS
KIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINT
LGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDI
NTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNIS
FWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYN
PDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDS
SSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSE
NELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALIS
YKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKA
VTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTI
DGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTG
WQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIIN
GKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGW
RIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGF
EYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTI
DGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEY
FAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTING
KKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYL
HDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYF
APANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDG
VIYFFGVDGVKAPGIYG

SEQ ID 37 - Toxin A-derived recombinant antigen - His-[helical spacer]-Thioredoxin- [thrombin site]-TxA4

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASDKIIHLTDDSFDTDVLK
ADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVA
ATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAK
SKNIPGLASISEDIKTLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLEN
VSDELYELKKLNNLDEKYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSII
TDVNGNLLDNIQLDHTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQL
VNLISNAVNDTINVLPTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVA
SIVGIGAEVTIFLLPIAGISAGIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVIS
EIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNA
PSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDT
RNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLI
KDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNI
IEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVF
MKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNL
FLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVV
VEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKV

NINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNF
KSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDIN
TGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYY
FDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAI
AFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDN
NSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIA
STGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDS
KAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVF
KGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEA
ATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFK
GPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVT
GWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIR
YQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFK
GSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAA
AGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 38 - Toxin A-derived recombinant antigen - His-Thioredoxin- [linear spacer]-[thrombin site]-TxA4

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALGGSGGSGGSGGSGG
SGGSGGSGGSGGSLVPRGSGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLL
LDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDE
KYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHT
SQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTI
TEGIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGIS
AGIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAM
EGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLR
SLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLS
YSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGN
QTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYN
YTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDN
NTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENIN
FVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSL
DFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTE
GSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGF
KIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYF
NNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNE
KYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDS
DCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYY
FNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNTDGI
MQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNP
NNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNN
NIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNT
AEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIE
GQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNTNTSIA
STGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNS
KAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIE
GQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVK
APGIYG

SEQ ID 39 - Toxin A-derived recombinant antigen - His-Thioredoxin-[helical spacer]-[thrombin site]-TxA4

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALLAEAAAKEAAAKEAAA
KEAAAKEAAAKAAAGGSLVPRGSGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIK
TLLLDASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNL
DEKYLISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLD
HTSQVNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVL
PTITEGIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIA
GISAGIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNI

LAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVP
GLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRN
KLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLII
GNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIA
YNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYV
DNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFE
NINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDIS
TSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWS
TEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHL
GFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHF
YFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIIN
NEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYF
DSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKK
YYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNT
DGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYY
FNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANT
HNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYF
NLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTH
NNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNT
NTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYF
GNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTD
ANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFG
VDGVKAPGIYG

SEQ ID 40 - Toxin A-derived recombinant antigen - His-[linear spacer]-Thioredoxin- [thrombin site]-TxA3

HHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASDKIIHLTDDSFDTDVLKADGA
ILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVG
ALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDF
NNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSR
VFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNF
IMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDV
LSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKI
NTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKD
DINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDN
ISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPI
YNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINL
DSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFN
SENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAA
LISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDND
SKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNG
YKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKA
VTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGY
TIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVT
GWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPN
GFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGW
QTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGF
EYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTI
NGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRF
LYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGF
EYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLF
EIDGVIYFFGVDGVKAPGIYG

SEQ ID 41 - Toxin A-derived recombinant antigen - His-[helical spacer]-Thioredoxin- [thrombin site]-TxA3

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASDKIIHLTDDSFDTDVLK
ADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVA
ATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAAPFTMESKKYGPLKTEDDKILVPIDDL
VISEIDFNNNSIKLGTCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMML
PNAPSRVFWWETGAVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKL

DKDTRNFIMPTITTNEIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIK
KGKLIKDVLSKIDINKNKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLIS
NLSNIIEKINTLGLDSKNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAE
DINVFMKDDINTITGKYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSN
FMNLFLDNISFWKLFGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNG
RNVVVEPIYNPDTGEDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTF
HKKVNINLDSSSFEYKWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYI
MSNFKSFNSENELDRDHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYY
FDINTGAALISYKIINGKHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGK
KYYFDNDSKAVTGWRIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDT
DTAIAFNGYKTIDGKHFYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYY
FDNNSKAVTGWQTIDSKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNT
AIASTGYTIINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGS
DSKAVTGWRIINNKKYYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTG
VFKGPNGFEYFAPANTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTA
EAATGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGV
FKGPNGFEYFAPANTHNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVA
VTGWQTINGKKYYFNTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQA
IRYQNRFLYLHDNIYYFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVF
KGSNGFEYFAPANTDANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMA
AAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 42 - Toxin A-derived recombinant antigen - His-Thioredoxin-[linear spacer]-[thrombin site]-TxA3

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALGGSGGSGGSGGSGG
SGGSGGSGGSGGSLVPRGSGSAAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNI
LAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVP
GLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRN
KLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLII
GNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIA
YNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYV
DNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFE
NINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDIS
TSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWS
TEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHL
GFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHF
YFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIIN
NEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYF
DSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKK
YYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYFNT
DGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYY
FNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANT
HNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYF
NLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTH
NNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYFNT
NTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYF
GNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTD
ANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFG
VDGVKAPGIYG

SEQ ID 43 - Toxin A-derived recombinant antigen - His-Thioredoxin-[helical spacer]-[thrombin site]-TxA3

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALLAEAAAKEAAAKEAAA
KEAAAKEAAAKAAAGGSLVPRGSGSAAAPFTMESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLG
TCNILAMEGGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETG
AVPGLRSLENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTN
EIRNKLSYSFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINK
NKLIIGNQTIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDS

KNIAYNYTDESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITG
KYYVDNNTDKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKL
FGFENINFVIDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTG
EDISTSLDFSYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEY
KWSTEGSDFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDR
DHLGFKIIDNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIING
KHFYFNNDGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGW
RIINNEKYYFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKH
FYFDSDCVVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTID
SKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAEAATGWQTIDGKKYYFNTNTAIASTGYTIINGKHFYF
NTDGIMQIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKK
YYFNPNNAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPA
NTHNNNIEGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKY
YFNLNTAEAATGWQTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPAN
THNNNIEGQAILYQNKFLTLNGKKYYFGSDSKAVTGLRTIDGKKYYFNTNTAVAVTGWQTINGKKYYF
NTNTSIASTGYTIISGKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIY
YFGNNSKAATGWVTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANT
DANNIEGQAIRYQNRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFF
GVDGVKAPGIYG

SEQ ID 44 - Toxin B-derived recombinant antigen - His-[linear spacer]-NusA-[thrombin site]-TxB4

HHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASNKEILAVVEAVSNEKALPRE
KIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDESLNLG
DYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLGNNAE
AVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARD
PGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPADVASIV
VDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTFTKYLD
IDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDNKPADD
LLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEASGALVP
RGSVTSLYKKAGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEE
KVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFISFEDIS
ETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAA
FFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIAT
IIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVN
NELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGS
GHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLE
NDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSF
YGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEI
NFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIP
YSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGY
YLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQS
NIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISST
VINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSN
DGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLG
LVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKN
YYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDG
EMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYF
AENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGS
KYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGI
VQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYY
FNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQ
IGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQ
LVISE.

SEQ ID 45 - Toxin B-derived recombinant antigen - His-NusA-[linear spacer]-[thrombin site]-TxB4

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL

FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALGGSGGSGGSGGSGGSGGSGGSGGSGGSLVPRGSGS
AAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNI
DTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKET
GESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKES
LSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELS
ETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVD
YFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAP
SITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYE
GEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYN
MGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSL
TFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFING
STKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDE
KTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIG
QFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVN
KVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVS
QVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMM
VSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFST
EDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGL
NQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDG
FKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLI
NDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAP
ANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIK
YYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQN
TLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE.

SEQ ID 46 - Toxin B-derived recombinant antigen - His-[helical spacer]- [thrombin site]-NusA-B4

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASNKEILAVVEAVSNEKA
LPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTFRRWLVVDEVTQPTKEITLEAARYEDES
LNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVVDQFREHEGEIITGVVKKVNRDNISLDLG
NNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKA
AARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTELGGERIDIVLWDDNPAQFVINAMAPAD
VASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSGWELNVMTVDDLQAKHQAEAHAAIDTF
TKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPTVEALRERAKNALATIAQAQEESLGDN
KPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADIEGLTDEKAGALIMAARNICWFGDEAS
GALVPRGSVTSLYKKAGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSS
DIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFI
SFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEV
NTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSE
GLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGI
PSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRM
EGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGL
RSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKL
SYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIIL
NSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSEL
QKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNV
NILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFV
NFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDS
GDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIR
YVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGL
IGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGE
TIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVE
WKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEID
GKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWK
DLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYF
YIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMEN

ESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFG
EDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYY
FDPDTAQLVISE

SEQ ID 47 - Toxin B-derived recombinant antigen - His-NusA-[helical spacer]- [thrombin site]-TxB4

HHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSGDFDTF
RRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAERAMVV
DQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEARGAQL
FVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARVQAVSTE
LGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRLASQLSG
WELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIEGLDEPT
VEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGIDDLADI
EGLTDEKAGALIMAARNICWFGDEASGALLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSLVPRG
SGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINV
ISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFIN
KETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSS
KESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIK
ELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATK
VVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFF
SAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRD
NYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLS
QYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNG
FVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENG
FINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTL
QDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGT
TSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGID
SCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEE
NKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINN
FGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQT
GVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGK
AFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVF
NTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEA
YIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDG
YKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACKGI
NLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKY
FAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 48 - Toxin B-derived recombinant antigen - His-[linear spacer]-Thioredoxin-[thrombin site]-TxB4

HHHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASDKIIHLTDDSFDTDVLKADG
AILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKV
GALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNL
PELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISD
ALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTV
NGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVE
LVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLG
IASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEI
DFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPN
APNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDS
NTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKG
DLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNS
NHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYY
SNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSD
SLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNR
QNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDAN
YINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPV
SEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDK
YYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDE
NIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYF

DDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIY
YFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSG
IIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGET
YTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQF
GYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIA
ATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 49 - Toxin B-derived recombinant antigen - His-Thioredoxin-[linear spacer] –[thrombin site]-TxB4

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALGGSGGSGGSGGSGG
SGGSGGSGGSGGSLVPRGSGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNN
SNSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELE
DSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDT
THEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLL
PTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLA
GISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKC
EIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETG
WTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTE
YIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIE
ENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGF
NSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITK
DNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIK
SIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDL
DDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDL
SIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYE
DGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAA
SIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRG
AVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGY
TEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVV
GWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDD
NYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYD
MENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMF
YFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGE
EYYFDPDTAQLVISE

SEQ ID 50 - Toxin B-derived recombinant antigen - His-[helical spacer]-Thioredoxin-[thrombin site]-TxB4

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASDKIIHLTDDSFDTDVLK
ADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVA
ATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAAPFTMSIIKDISSKEYISFNPKENKITVK
SKNLPELSTLLQEIRNNSNSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIE
SISDALCDLKQQNELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTI
FDTVNGKLVKKVNLDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAA
KVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIIT
SSLGIASGFSILLVPLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDL
VISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLM
VLPNAPNRVFAWETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRI
NLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESD
KIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKI
LMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKP
SFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGN
TNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTL
YVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVI
VLDANYINEKINVNINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDK
QDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVT
VGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTG
KLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSIND
NKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILN
FNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVF

YFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDV
YYFGETYTIETGWIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNE
NGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYY
FTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 51 - Toxin B-derived recombinant antigen - His-Thioredoxin–[Helical spacer]-[thrombin site]-TxB4

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALLAEAAAKEAAAKEAAA
KEAAAKEAAAKAAAGGSLVPRGSGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEI
RNNSNSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQN
ELEDSHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVN
LDTTHEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETI
DLLPTLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLV
PLAGISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVL
GKCEIWRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAW
ETGWTPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPII
TTEYIREKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILST
LSIEENKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKID
YIGFNSELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVK
VITKDNVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLES
MNIKSIFVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEP
NYDLDDSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINV
NINDLSIRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTP
SYYEDGLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPING
GAASIGETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDN
YRGAVEWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMK
VGYTEIDGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFT
AVVGWKDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQ
NIDDNYFYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETG
WIYDMENESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIE
DKMFYFGEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVI
IDGEEYYFDPDTAQLVISE

SEQ ID 52 - Toxin B-derived recombinant antigen - His-[helical spacer]-Thioredoxin -[thrombin site]-TxB3

HHHHHHHHHHGGSLAEAAAKEAAAKEAAAKEAAAKEAAAKAAAGGSHMASDKIIHLTDDSFDTDVLK
ADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVA
ATKVGALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAGGSMPQDDLVISEIDFNNNSIVLGKCEI
WRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGW
TPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYI
REKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEE
NKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFN
SELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKD
NVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSI
FVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLD
DSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLS
IRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYED
GLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASI
GETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAV
EWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEI
DGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGW
KDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNY
FYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDME
NESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYF
GEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEY
YFDPDTAQLVISE

SEQ ID 53 - Toxin B-derived recombinant antigen - His-Thioredoxin-[helical spacer]- [thrombin site]-TxB3

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALLAEAAAKEAAAKEAAA
KEAAAKEAAAKAAAGGSLVPRGSGSAAGGSMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVT
DDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTK
LLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGG
TYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGE
VNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDS
EGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDI
KISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILD
ANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQ
KYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFI
LMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYN
EKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQ
SGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYF
SPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGE
MQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDE
DTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVF
DTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETK
KACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNT
PDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 54 - Toxin B-derived recombinant antigen - His-[linear spacer]-Thioredoxin-[thrombin site]-TxB3

HHHHHHHHHGGSGGSGGSGGSGGSGGSGGSGGSGGSGGSHMASDKIIHLTDDSFDTDVLKADGA
ILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVG
ALSKGQLKEFLDANLARALVPRGSVTSLYKKAGSAAGGSMPQDDLVISEIDFNNNSIVLGKCEIWRME
GGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLR
SLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLS
YSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILN
SHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQ
KNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNI
LTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNF
LQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDI
SSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYV
WSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIG
YDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETII
DDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWK
ELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGK
HFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLE
DGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDD
NGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDK
YYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGV
MQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPD
TAQLVISE

SEQ ID 55 - Toxin B-derived recombinant antigen - His-Thioredoxin-[linear spacer]-[thrombin site]-TxB3

HHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLTVAKL
NIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALSKGQLKEFLDANLARALGGSGGSGGSGGSGG
SGGSGGSGGSGGSLVPRGSGSAAGGSMPQDDLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDI
DHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGWTPGLRSLENDGTKLLD
RIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYIREKLSYSFYGSGGTYA
LSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEENKIILNSHEINFSGEVNG
SNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFNSELQKNIPYSFVDSEGK
ENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKDNVNILTGYYLKDDIKISL
SLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSIFVNFLQSNIKFILDANFII
SGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLDDSGDISSTVINFSQKYLY
GIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLSIRYVWSNDGNDFILMST
SEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYEDGLIGYDLGLVSLYNEKFY
INNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASIGETIIDDKNYYFNQSGVL

QTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAVEWKELDGEMHYFSPET
GKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEIDGKHFYFAENGEMQIG
VFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGWKDLEDGSKYYFDEDTAE
AYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNYFYIDDNGIVQIGVFDTSD
GYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDMENESDKYYFNPETKKACK
GINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYFGEDGVMQIGVFNTPDGF
KYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEYYFDPDTAQLVISE

SEQ ID 56 - Toxin B-derived recombinant antigen - His-NusA-[Intein A sequence]-TxB4-His
MGSSHHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSG
DFDTFRRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAER
AMVVDQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEA
RGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARV
QAVSTELGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRL
ASQLSGWELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIE
GLDEPTVEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGI
DDLADIEGLTDEKAGALIMAARNICWFGDEASGALRTRVKVVKNKALAEGTRIFDPVTGTTHRIEDVVD
GRKPIHVVAAAKDGTLHARPVVSWFDQGTRDVIGLRIAGGAILWATPDHKVLTEYGWRAAGELRKGD
RVAQPRRFDGFGDSAPIPARVQALADALDDKFLHDMLAEELRYSVIREVLPTRRARTFGLEVEELHTL
VAEGVVVHNSSPPFKQAEFGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNS
NSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELED
SHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTT
HEVNTLNAAFFIQSLIEYNSSKESLSNLSVAMKVQVYAQLFSTGLNTITDAAKVVELVSTALDETIDLLP
TLSEGLPIIATIIDGVSLGAAIKELSETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAG
ISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKIMMPQDDLVISEIDFNNNSIVLGKCEI
WRMEGGSGHTVTDDIDHFFSAPSITYREPHLSIYDVLEVQKEELDLSKDLMVLPNAPNRVFAWETGW
TPGLRSLENDGTKLLDRIRDNYEGEFYWRYFAFIADALITTLKPRYEDTNIRINLDSNTRSFIVPIITTEYI
REKLSYSFYGSGGTYALSLSQYNMGINIELSESDVWIIDVDNVVRDVTIESDKIKKGDLIEGILSTLSIEE
NKIILNSHEINFSGEVNGSNGFVSLTFSILEGINAIIEVDLLSKSYKLLISGELKILMLNSNHIQQKIDYIGFN
SELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKD
NVNILTGYYLKDDIKISLSLTLQDEKTIKLNSVHLDESGVAEILKFMNRKGNTNTSDSLMSFLESMNIKSI
FVNFLQSNIKFILDANFIISGTTSIGQFEFICDENDNIQPYFIKFNTLETNYTLYVGNRQNMIVEPNYDLD
DSGDISSTVINFSQKYLYGIDSCVNKVVISPNIYTDEINITPVYETNNTYPEVIVLDANYINEKINVNINDLS
IRYVWSNDGNDFILMSTSEENKVSQVKIRFVNVFKDKTLANKLSFNFSDKQDVPVSEIILSFTPSYYED
GLIGYDLGLVSLYNEKFYINNFGMMVSGLIYINDSLYYFKPPVNNLITGFVTVGDDKYYFNPINGGAASI
GETIIDDKNYYFNQSGVLQTGVFSTEDGFKYFAPANTLDENLEGEAIDFTGKLIIDENIYYFDDNYRGAV
EWKELDGEMHYFSPETGKAFKGLNQIGDYKYYFNSDGVMQKGFVSINDNKHYFDDSGVMKVGYTEI
DGKHFYFAENGEMQIGVFNTEDGFKYFAHHNEDLGNEEGEEISYSGILNFNNKIYYFDDSFTAVVGW
KDLEDGSKYYFDEDTAEAYIGLSLINDGQYYFNDDGIMQVGFVTINDKVFYFSDSGIIESGVQNIDDNY
FYIDDNGIVQIGVFDTSDGYKYFAPANTVNDNIYGQAVEYSGLVRVGEDVYYFGETYTIETGWIYDME
NESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYF
GEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEY
YFDPDTAQLVISEGHHHHHH

SEQ ID 57 - Toxin B-derived recombinant antigen - His-NusA-[Intein BT sequence]-TxB4-His
MGSSHHHHHHSHMASNKEILAVVEAVSNEKALPREKIFEALESALATATKKKYEQEIDVRVQIDRKSG
DFDTFRRWLVVDEVTQPTKEITLEAARYEDESLNLGDYVEDQIESVTFDRITTQTAKQVIVQKVREAER
AMVVDQFREHEGEIITGVVKKVNRDNISLDLGNNAEAVILREDMLPRENFRPGDRVRGVLYSVRPEA
RGAQLFVTRSKPEMLIELFRIEVPEIGEEVIEIKAAARDPGSRAKIAVKTNDKRIDPVGACVGMRGARV
QAVSTELGGERIDIVLWDDNPAQFVINAMAPADVASIVVDEDKHTMDIAVEAGNLAQAIGRNGQNVRL
ASQLSGWELNVMTVDDLQAKHQAEAHAAIDTFTKYLDIDEDFATVLVEEGFSTLEELAYVPMKELLEIE
GLDEPTVEALRERAKNALATIAQAQEESLGDNKPADDLLNLEGVDRDLAFKLAARGVCTLEDLAEQGI
DDLADIEGLTDEKAGALIMAARNICWFGDEASGALEVFGEFGSGKAFARDTEVYYENDTVPHMESIEE
MYSKYASMNGELPFDNGYAVPLDNVFVYTLDIASGEIKKTRASYIYREKVEKLIEIKLSSGYSLKVTPSH
PVLLFRDGLQWVPAAEVKPGDVVVGVREEVLRRRIISKGELEFHEVSSVRIIDYNNWVYDLVIPETHNF
IAPNGLVLHNTQLAHTLAVMGSAAAPFTMSIIKDISSKEYISFNPKENKITVKSKNLPELSTLLQEIRNNS
NSSDIELEEKVMLTECEINVISNIDTQIVEERIEEAKNLTSDSINYIKDEFKLIESISDALCDLKQQNELED
SHFISFEDISETDEGFSIRFINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVNLDTT
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SELQKNIPYSFVDSEGKENGFINGSTKEGLFVSELPDVVLISKVYMDDSKPSFGYYSNNLKDVKVITKD
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NESDKYYFNPETKKACKGINLIDDIKYYFDEKGIMRTGLISFENNNYYFNENGEMQFGYINIEDKMFYF
GEDGVMQIGVFNTPDGFKYFAHQNTLDENFEGESINYTGWLDLDEKRYYFTDEYIAATGSVIIDGEEY
YFDPDTAQLVISEGHHHHHH

SEQ ID 58 - Toxin A-derived recombinant antigen (TxA4; residues 770-2710) expression construct

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DARAKAQFEEYKRNYFEGAGGSIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLDASVSP
DTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYLISFEDI
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AAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITEGIPIVS
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NNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAMEGGSG
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GGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQTIDFSG
DIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYTDESNN
KYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNTDKSIDF
SISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFVIDKYFT
LVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDFSYEPLY
GIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGSDFILVRY
LEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKIIDNKTYYY
DEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNNDGVMQL
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FSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFNTNTAEA
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PNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPNNAIAAIHL
CTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNIEGQAIVY
QNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTAEAATGW
QTIDGKKYYFNTNTFIASTGYTSINGKHFYFNTDGIMQIGVFKGPNGFEYFAPANTHNNNIEGQAILYQ
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GKHFYFNTDGIMQIGVFKGPDGFEYFAPANTDANNIEGQAIRYQNRFLYLHDNIYYFGNNSKAATGW
VTIDGNRYYFEPNTAMGANGYKTIDNKNFYFRNGLPQIGVFKGSNGFEYFAPANTDANNIEGQAIRYQ
NRFLHLLGKIYYFGNNSKAVTGWQTINGKVYYFMPDTAMAAAGGLFEIDGVIYFFGVDGVKAPGIYG

SEQ ID 59 - Toxin A-derived recombinant antigen (TxA4 truncate; residues 770-2389) expression construct

MGSSHHHHHHSHMASDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIAPILDEIADEYQGKLT
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RAKAQFEEYKRNYFEGAGGSAAAPFTMIMSDLSSKEYIFFDSIDNKLKAKSKNIPGLASISEDIKTLLLD
ASVSPDTKFILNNLKLNIESSIGDYIYYEKLEPVKNIIHNSIDDLIDEFNLLENVSDELYELKKLNNLDEKYL

ISFEDISKNNSTYSVRFINKSNGESVYVETEKEIFSKYSEHITKEISTIKNSIITDVNGNLLDNIQLDHTSQ
VNTLNAAFFIQSLIDYSSNKDVLNDLSTSVKVQLYAQLFSTGLNTIYDSIQLVNLISNAVNDTINVLPTITE
GIPIVSTILDGINLGAAIKELLDEHDPLLKKELEAKVGVLAINMSLSIAATVASIVGIGAEVTIFLLPIAGISA
GIPSLVNNELILHDKATSVVNYFNHLSESKKYGPLKTEDDKILVPIDDLVISEIDFNNNSIKLGTCNILAME
GGSGHTVTGNIDHFFSSPSISSHIPSLSIYSAIGIETENLDFSKKIMMLPNAPSRVFWWETGAVPGLRS
LENDGTRLLDSIRDLYPGKFYWRFYAFFDYAITTLKPVYEDTNIKIKLDKDTRNFIMPTITTNEIRNKLSY
SFDGAGGTYSLLLSSYPISTNINLSKDDLWIFNIDNEVREISIENGTIKKGKLIKDVLSKIDINKNKLIIGNQ
TIDFSGDIDNKDRYIFLTCELDDKISLIIEINLVAKSYSLLLSGDKNYLISNLSNIIEKINTLGLDSKNIAYNYT
DESNNKYFGAISKTSQKSIIHYKKDSKNILEFYNDSTLEFNSKDFIAEDINVFMKDDINTITGKYYVDNNT
DKSIDFSISLVSKNQVKVNGLYLNESVYSSYLDFVKNSDGHHNTSNFMNLFLDNISFWKLFGFENINFV
IDKYFTLVGKTNLGYVEFICDNNKNIDIYFGEWKTSSSKSTIFSGNGRNVVVEPIYNPDTGEDISTSLDF
SYEPLYGIDRYINKVLIAPDLYTSLININTNYYSNEYYPEIIVLNPNTFHKKVNINLDSSSFEYKWSTEGS
DFILVRYLEESNKKILQKIRIKGILSNTQSFNKMSIDFKDIKKLSLGYIMSNFKSFNSENELDRDHLGFKII
DNKTYYYDEDSKLVKGLININNSLFYFDPIEFNLVTGWQTINGKKYYFDINTGAALISYKIINGKHFYFNN
DGVMQLGVFKGPDGFEYFAPANTQNNNIEGQAIVYQSKFLTLNGKKYYFDNDSKAVTGWRIINNEKY
YFNPNNAIAAVGLQVIDNNKYYFNPDTAIISKGWQTVNGSRYYFDTDTAIAFNGYKTIDGKHFYFDSDC
VVKIGVFSTSNGFEYFAPANTYNNNIEGQAIVYQSKFLTLNGKKYYFDNNSKAVTGWQTIDSKKYYFN
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QIGVFKGPNGFEYFAPANTDANNIEGQAILYQNEFLTLNGKKYYFGSDSKAVTGWRIINNKKYYFNPN
NAIAAIHLCTINNDKYYFSYDGILQNGYITIERNNFYFDANNESKMVTGVFKGPNGFEYFAPANTHNNNI
EGQAIVYQNKFLTLNGKKYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATGWQTIDGKKYYFNLNTA
EAATGWQTIDGKKYYFNTNTFIAST

CLAIMS

1. A fusion protein, consisting of or comprising a first amino acid sequence and a second amino acid sequence, wherein:

    a) the first amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 874-1850 of a *C. difficile* Toxin A sequence or residues 1444-1851 of a *C. difficile* Toxin B sequence; and

    b) the second amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of a long repeat unit located within amino acid residues 1851-2710 of a *C. difficile* Toxin A sequence or within amino acid residues 1852-2366 of a *C. difficile* Toxin B sequence;

    wherein said *C. difficile* Toxin A sequences have the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 3, and said *C. difficile* Toxin B sequences have the amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4;

    wherein the fusion protein is immunogenic;

    wherein the fusion protein lacks cysteine protease activity, wherein part or all of the amino acid sequence providing said activity is absent;

    with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2710 of a *C. difficile* Toxin A; and

    with the proviso that the fusion protein is not a polypeptide comprising amino acid residues 543-2366 of a *C. difficile* Toxin B.

2. The fusion protein according to Claim 1, wherein the first amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 770-1850 or 774-1850 or 794-1850 or 814-1850 or 834-1850 or 854-1850 of a *C. difficile* Toxin A sequence; or residues 1164-1851 or 1204-1851 or 1244-1851 or 1284-1851 or 1324-1851 or 1364-1851 of a *C. difficile* Toxin B sequence.

3. The fusion protein according to Claim 1, wherein the first amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid

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sequence consisting of residues 770-1850 of a *C. difficile* Toxin A sequence or residues 767-1851 of a *C. difficile* Toxin B sequence.

4. The fusion protein according to any one of Claims 1 to 3, wherein the second amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence selected from the amino acid sequences 1851-2007, 1851-2141, 1851-2253, 1851-2389, 1851-2502, 1851-2594, and 1851-2710 of a *C. difficile* Toxin A sequence.

5. The fusion protein according to any one of Claims 1 to 3, wherein the second amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence selected from one or more of the amino acid sequences 1851-2389, 1851-2007, 2008-2141, 2142-2253, 2254-2389, 2390-2502, 2503-2594, and 2595-2710 of a *C. difficile* Toxin A sequence.

6. The fusion protein according to any one of Claims 1 to 3, wherein the second amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence selected from amino acid sequences 1852-2007, 1852-2139, 1852-2273, and 1852-2366 of a *C. difficile* Toxin B sequence.

7. The fusion protein according to any one of Claims 1 to 3, wherein the second amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence selected from amino acid sequences 1852-2007, 2008-2139, 2140-2273, and 2274-2366 of a *C. difficile* Toxin B sequence.

8. The fusion protein according to any one of Claims 1 to 3, wherein the first amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 874-1850 of a *C. difficile* Toxin A sequence or residues 1444-1851 of a *C. difficile* Toxin B sequence, and wherein the second amino acid sequence is an amino acid sequence that has at least 80% sequence identity with an amino acid sequence consisting of residues 1851-2710 of a *C. difficile* Toxin A sequence or residues 1852-2366 of a *C. difficile* Toxin B sequence.

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9. The fusion protein according to any one of Claims 1 to 8, wherein said fusion protein does not include any Toxin A and/or Toxin B amino sequence in addition to said first amino acid sequence and/or said second amino acid sequence.

10. Use of the fusion protein according to any one of Claims 1 to 9 for immunizing an animal to induce production of an antibody specific for one or more of *C. difficile* Toxin A and *C. difficile* Toxin B.

11. An *in vitro* method for isolating antibodies that bind to *C. difficile* Toxin A and/or to *C. difficile* Toxin B, said method comprising

    a) immobilising on a surface one or more *C. difficile* fusion protein(s) according to any one of Claims 1 to 9;

    b) contacting the immobilised fusion protein(s) with a solution containing antibodies that bind to *C. difficile* Toxin A and/or Toxin B;

    c) allowing said antibodies to bind to said fusion protein(s), thereby forming a bound complex of antibody and fusion protein(s);

    d) washing away any unbound antibody or protein; and

    e) eluting the bound antibodies from the surface, thereby providing affinity-purified antibodies.

12. The method according to Claim 11, wherein the surface is a matrix within a column.

13. Polyclonal antibodies that bind to and neutralise a *C. difficile* Toxin A and/or a *C. difficile* Toxin B, wherein said antibodies are obtained by a method according to Claim 11 or 12 and wherein said polyclonal antibodies comprise antibodies that do not substantially bind to an effector domain and/or to a cysteine protease domain of a *C. difficile* Toxin A or Toxin B; and wherein said antibodies bind to an epitope presented by residues 1500-1850 of a *C. difficile* Toxin A and/or by residues 1500-1851 of a *C. difficile* Toxin B.

14. The antibodies according to Claim 13, wherein said antibodies comprise whole IgG, Fab antibodies or $F(ab')_2$ antibodies.

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15. The fusion protein according to any one of Claims 1 to 9 or the polyclonal antibodies according to Claim 13 or 14, for use in the prevention, treatment or suppression of *C. difficile* infection (CDI) in a subject.

16. The fusion protein according to Claim 15, for use in the prevention, treatment or suppression of CDI in a human.

17. A therapeutically effective amount of the fusion protein according to any one of Claims 1 to 9 or the polyclonal antibodies according to Claim 13 or 14, for preventing, treating or suppressing CDI in a human.

18. The therapeutically effective amount of the fusion protein according to Claim 17, wherein said fusion protein is for use as part of a combination therapy with one or more additional antigens selected from the group consisting of a *C. difficile* non-Toxin antigen, an inactivated or attenuated whole cell *C. difficile* bacterium, a *C. difficile* cell extract, an antigen from a bacterium that causes nosocomial infection, and an inactivated or attenuated whole cell bacterium that causes nosocomial infection.

19. The therapeutically effective amount of the fusion protein according to Claim 18, wherein said combination therapy further comprises one or more antibiotics.

20. The therapeutically effective amount of the fusion protein according to Claim 19, wherein the one or more antibiotics is an antibiotic effective against a bacterium that causes nosocomial infection.

21. The therapeutically effective amount of the polyclonal antibodies according to Claim 17, wherein said polyclonal antibodies are for use as part of a combination therapy with one or more additional antibodies selected from the group consisting of an antibody that binds to a *C. difficile* non-Toxin antigen, an antibody that binds to a whole cell *C. difficile* bacterium, an antibody that binds to antigen from a bacterium that causes nosocomial infection, and an antibody that binds to a whole cell bacterium that causes nosocomial infection.

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22. The therapeutically effective amount of the polyclonal antibodies according to Claim 21, wherein the combination therapy further comprises one or more antibiotics.

23. The therapeutically effective amount of the polyclonal antibodies according to Claim 22, wherein the one or more antibiotics is an antibiotic effective against a bacterium that causes nosocomial infection.

24. Use of the polyclonal antibodies according to Claim 13 or 14 in an *in vitro* immunoassay method for confirming the presence or absence of a *C. difficile* infection in a patient sample, wherein the presence of a *C. difficile* infection is confirmed by detecting the binding of said antibody to a *C. difficile* Toxin A and/or Toxin B present in said sample, and wherein failure to detect the binding of said antibody to a *C. difficile* Toxin A and/or Toxin B present in said sample confirms the absence of a *C. difficile* infection.

25. Use of the fusion protein according to any one of Claims 1 to 9 in an *in vitro* immunoassay method for confirming the presence or absence of a *C. difficile* infection in a patient sample, wherein the presence of a *C. difficile* infection is confirmed by detecting the binding of said fusion protein to an antibody present in said sample, and wherein failure to detect the binding of said fusion protein to an antibody present in said sample confirms the absence of a *C. difficile* infection.

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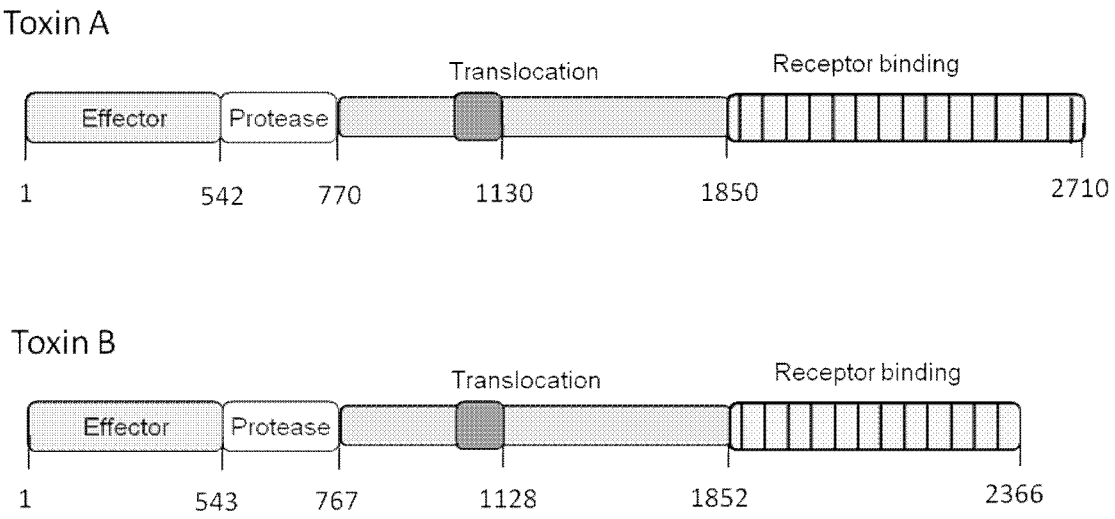
Figure 1


Toxin A
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Receptor binding
Effector
Protease
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1130
1850
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Toxin B
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Receptor binding
Effector
Protease
1
543
767
1128
1852
2366

Figure 2

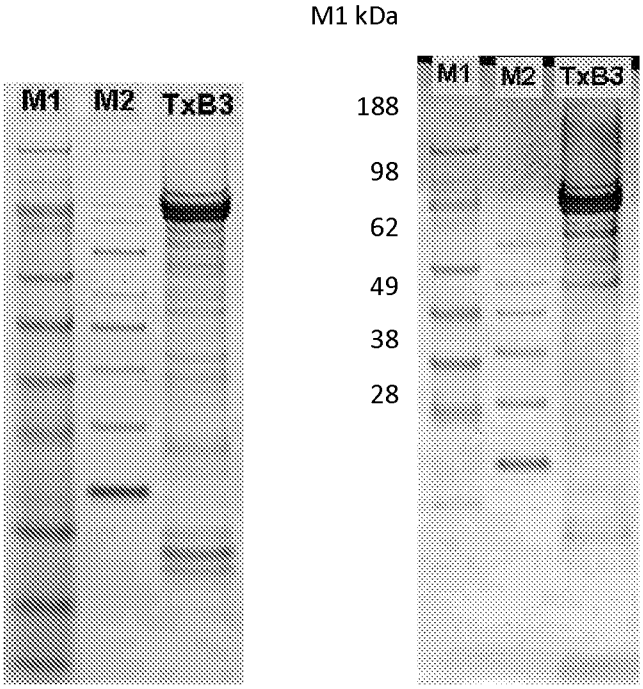

Figure 3

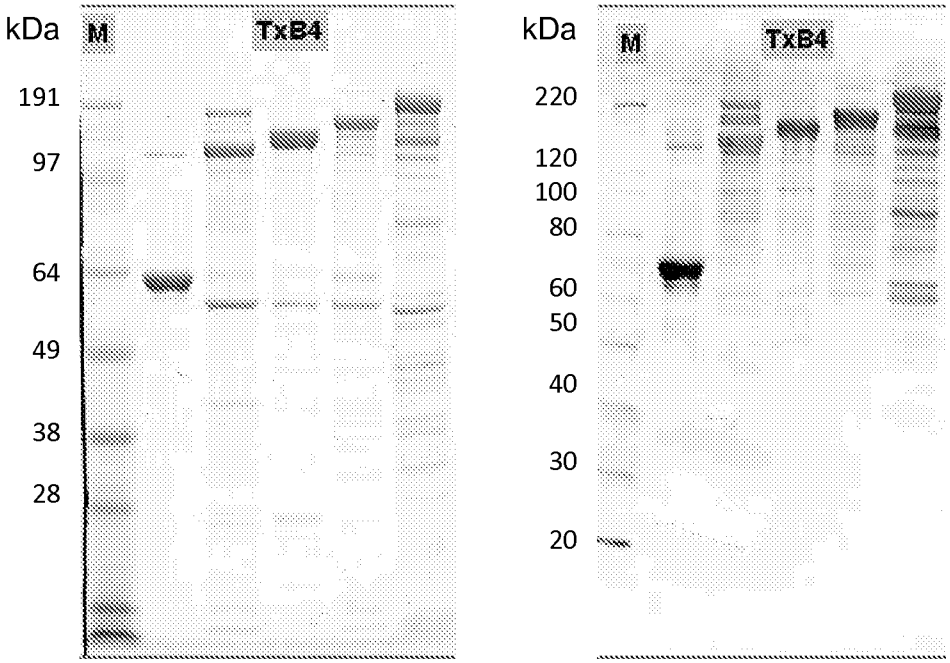
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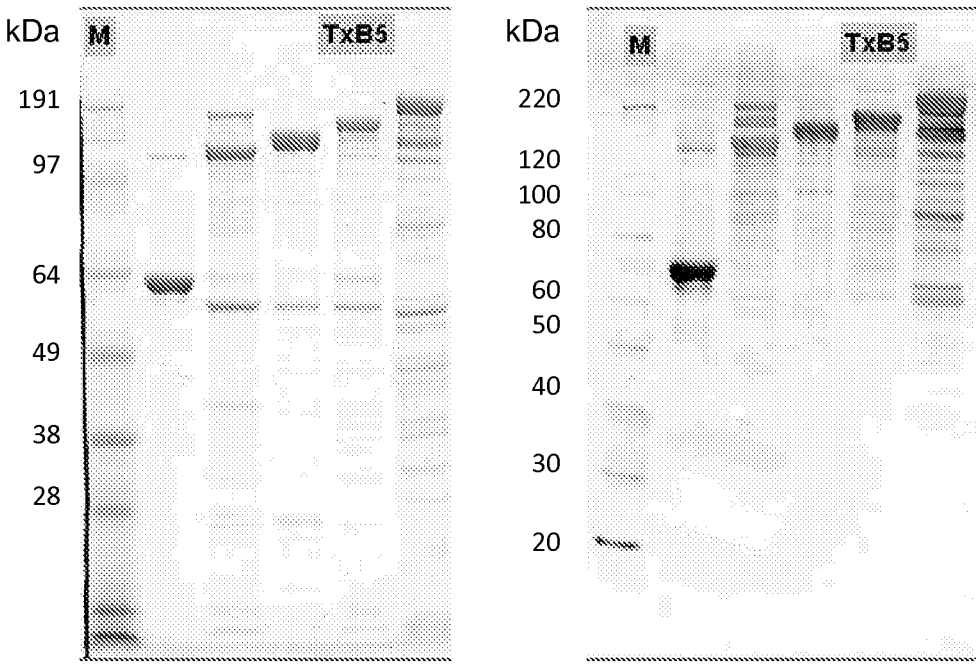
Figure 4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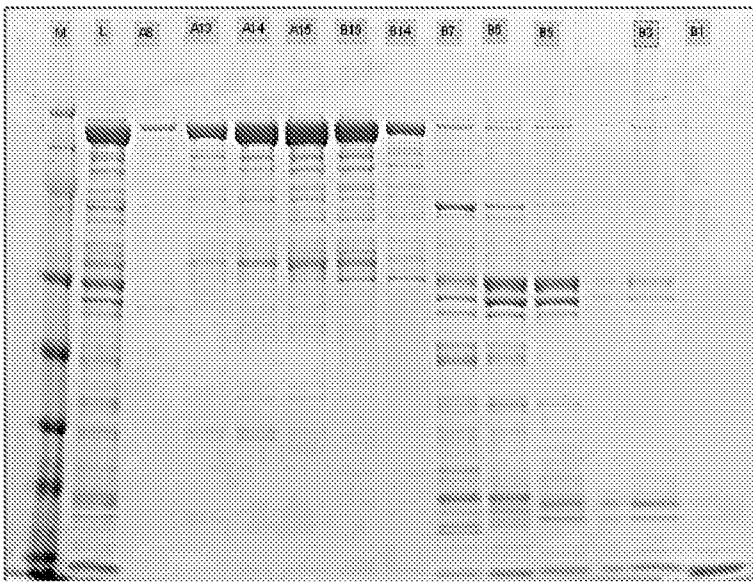
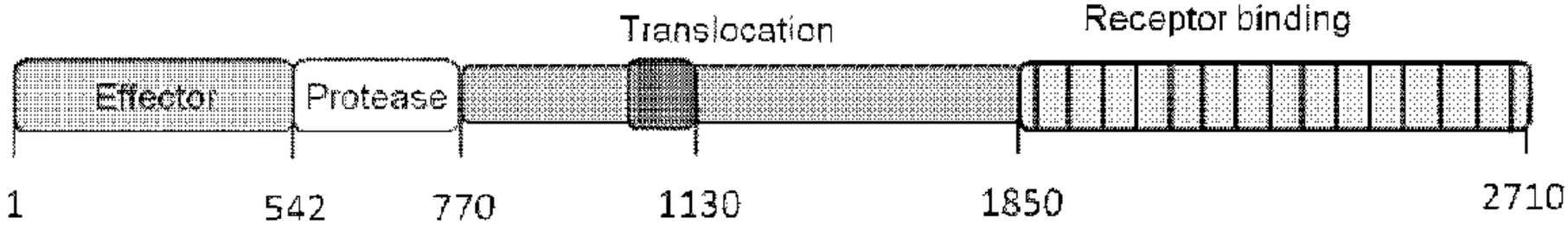
5/5

Figure 5



Toxin A



Toxin B