



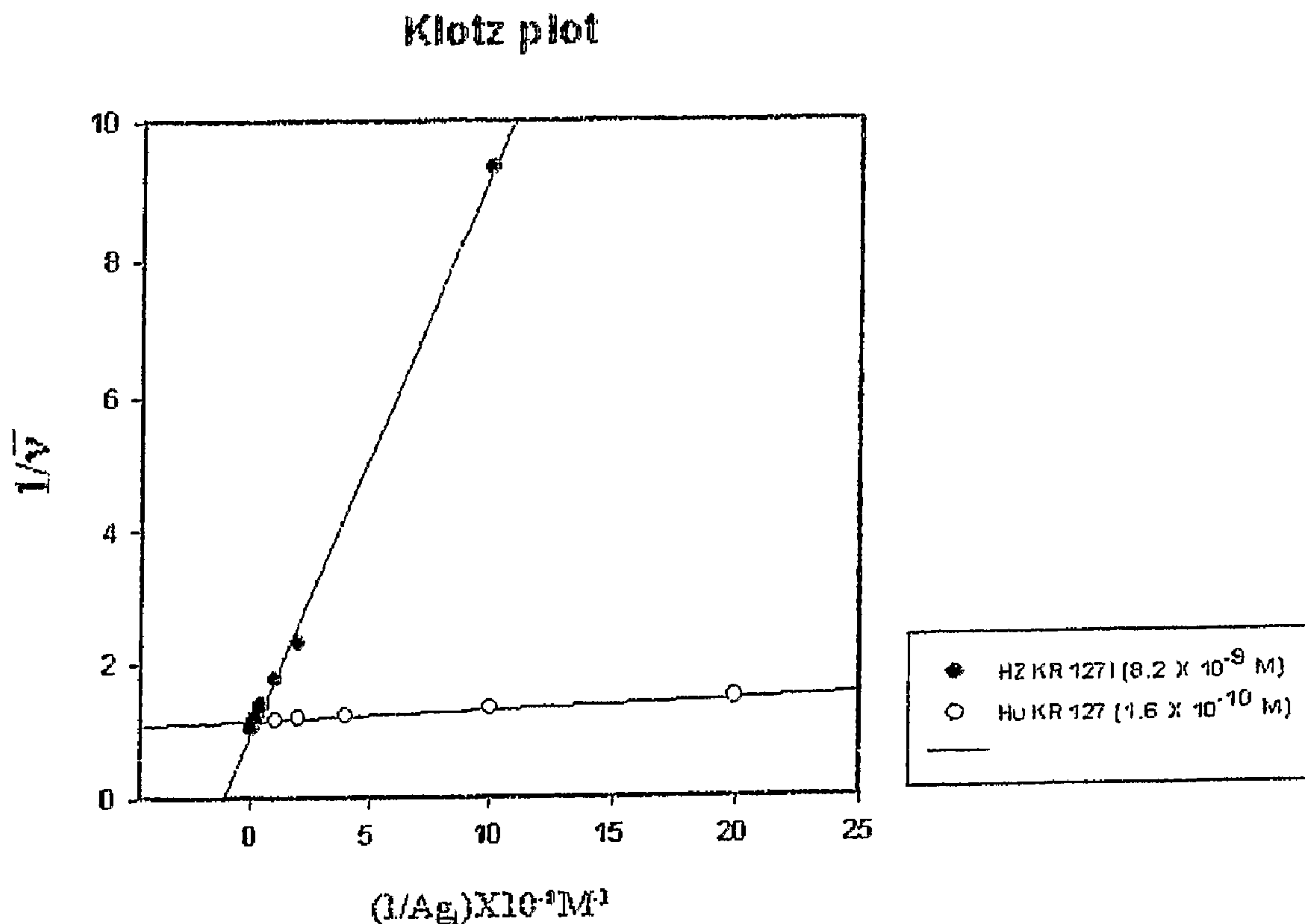
(86) Date de dépôt PCT/PCT Filing Date: 2003/03/22
(87) Date publication PCT/PCT Publication Date: 2003/10/02
(45) Date de délivrance/Issue Date: 2012/04/17
(85) Entrée phase nationale/National Entry: 2005/01/13
(86) N° demande PCT/PCT Application No.: KR 2003/000564
(87) N° publication PCT/PCT Publication No.: 2003/080672
(30) Priorité/Priority: 2002/03/22 (KR10-2002-0015708)

(51) Cl.Int./Int.Cl. *C12N 15/13* (2006.01),
A61K 39/395 (2006.01), *C07K 16/08* (2006.01),
C07K 16/46 (2006.01), *C12N 1/21* (2006.01),
C12N 15/00 (2006.01), *C12N 15/70* (2006.01)

(72) Inventeurs/Inventors:
HONG, HYO JEONG, KR;
MAENG, CHEOL-YOUNG, KR;
YANG, GI-HYEOK, KR;
JANG, MEONG HEE, KR;
OH, MEE SOOK, KR;
SONG, JIN-SOO, KR;
JANG, YOUNG KUG, KR

(73) Propriétaire/Owner:

(54) Titre : ANTICORPS HUMANISE ET PROCEDE DE PREPARATION CORRESPONDANT
(54) Title: HUMANIZED ANTIBODY AND PROCESS FOR PREPARING SAME



(57) Abrégé/Abstract:

A humanized antibody is produced by process comprising the steps of: (a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions; and (b) grafting said SDR to at least one of the corresponding amino acid sequences in human antibody variable regions.

(73) Propriétaires(suite)/Owners(continued):APROGEN, INC., KR

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 October 2003 (02.10.2003)

PCT

(10) International Publication Number
WO 03/080672 A1

(51) International Patent Classification⁷: **C07K 16/18**

(21) International Application Number: PCT/KR03/00564

(22) International Filing Date: 22 March 2003 (22.03.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10-2002-0015708 22 March 2002 (22.03.2002) KR

(71) Applicant (for all designated States except US): **APROGEN, INC.** [KR/KR]; Korea Research Institute of Bioscience and Biotechnology, 52, Oeun-dong, Yuseong-gu, Daejeon 305-333 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HONG, Hyo, Jeong** [KR/KR]; 117-201 Crova Apt., Dunsan-1-dong, Seo-gu, Daejeon 302-772 (KR). **MAENG, Cheol-Young** [KR/KR]; 176-15 Yongjeon-dong, Dong-gu, Daejeon 300-200 (KR). **YANG, Gi-Hyeok** [KR/KR]; 104-2101 Dule Hyundai Apt., 895, Sinbang-dong, Cheonan-si,

Chungcheongnam-do 330-771 (KR). **JANG, Meong, Hee** [KR/KR]; 411-95 Sambu Apt., Taepyung-dong, Jung-gu, Daejeon 301-773 (KR). **OH, Mee, Sook** [KR/KR]; 197-6 Jayang-dong, Dong-gu, Daejeon 300-100 (KR). **SONG, Jin-Soo** [KR/KR]; 167-16 Doma-2-dong, Seo-gu, Daejeon 302-162 (KR). **JANG, Young, Kug** [KR/KR]; 101-1514 Chowon Apt., Mannyun-dong, Seo-gu, Daejeon 302-740 (KR).

(74) Agents: **JANG, Seongku** et al.; 19th Fl., KEC Building, 275-7 Yangjae-dong, Seocho-ku, Seoul 137-130 (KR).

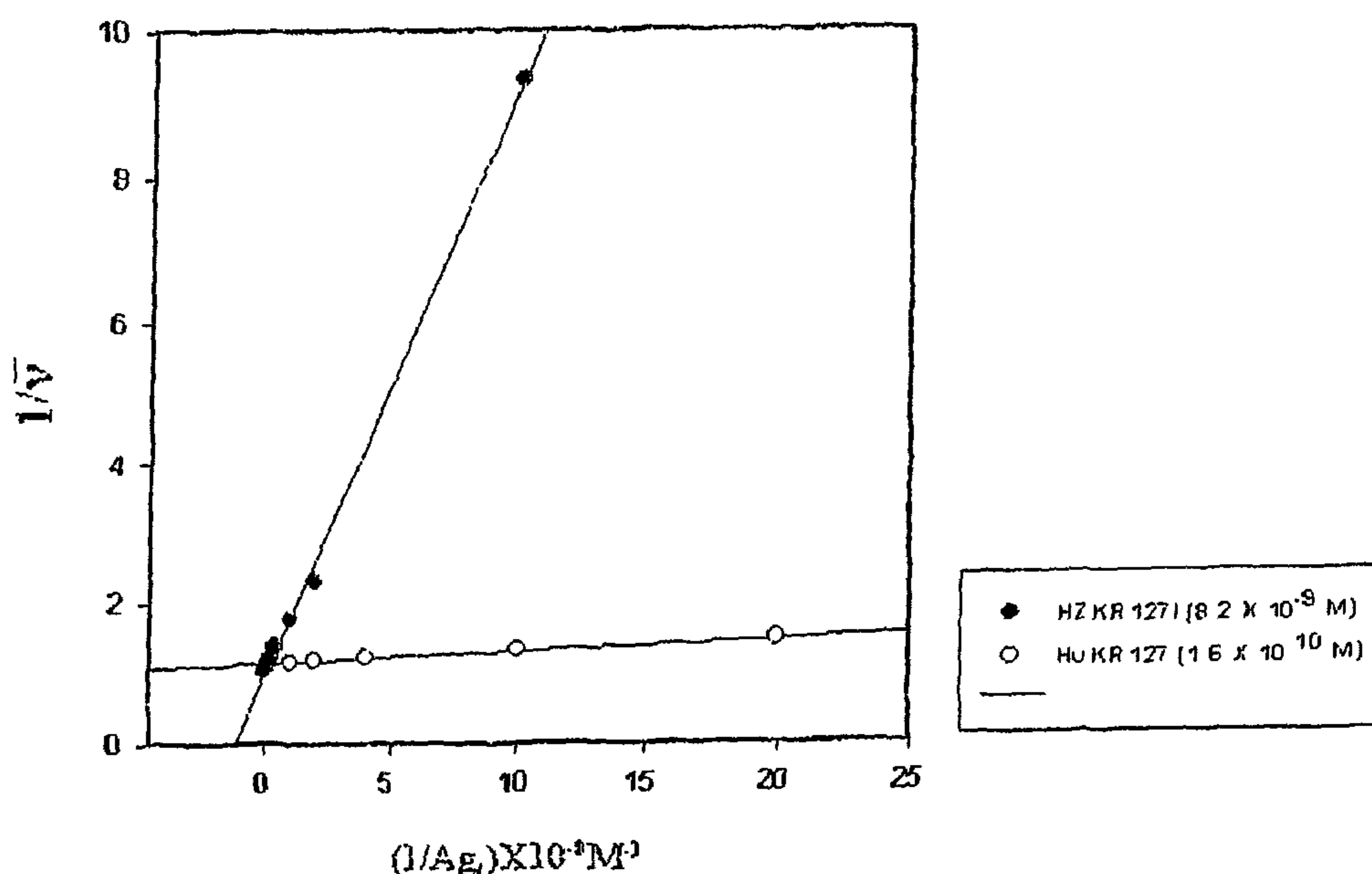
(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,

[Continued on next page]

(54) Title: HUMANIZED ANTIBODY AND PROCESS FOR PREPARING SAME

Klotz plot



(57) Abstract: A humanized antibody is produced by process comprising the steps of: (a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions; and (b) grafting said SDR to at least one of the corresponding amino acid sequences in human antibody variable regions.

WO 03/080672 A1

WO 03/080672 A1



ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *with international search report*

HUMANIZED ANTIBODY AND PROCESS FOR PREPARING SAME

Field of the Invention

5 The present invention relates to a process for preparing a humanized antibody by grafting SDRs (specificity determining residues) in CDRs (complementary determining residues) of murine monoclonal antibody to human antibody and the humanized antibody prepared according to said process.

Background of the Invention

10 For preventing infectious diseases such as hepatitis B, there has generally been used a method of administering immunoglobulins formed in blood plasma against a target antigen. However, the method has the
15 problems that the immunoglobulins generally have low specificity and may contain contaminants.

Murine monoclonal antibody derived from mouse has been reported to have high affinity to antigen and is suitable for mass-production. However, repeated injection of murine monoclonal antibody induces an
20 immune response because the murine antibody is regarded as a foreign antigen in humans (Shawler D.L. et al., *J. Immunol.*, 135, 1530-1535(1985)).

Accordingly, numerous efforts have been made to generate "humanized antibody" by: grafting the CDR (complementarity determining region) of murine monoclonal antibody variable region which directly binds
25 to antigens, to a human antibody framework (CDR-grafting method); and replacing the amino acid residues of the human antibody framework region (FR) that influence the CDR conformation with the amino acid residues of murine monoclonal antibody. The humanized antibody thus obtained maintains the affinity and specificity of original murine monoclonal antibody,
30 and minimizes HAMA(human anti-mouse antibody) response in humans (Riechmann et al., *Nature*, 332, 323-327(1988); Queen C. et al., *Proc. Natl. Acad. Sci. USA*, 86, 10029-10033(1989); Nakatani et al., *Protein Engineering*, 7, 435-443(1994)). However, this humanized antibody still causes problems when injected repeatedly into humans (Stephens et al.,
35 *Immunology*, 85, 668-674(1995); Sharkey et al., *Cancer Research*, 55, 5935s-5945s(1995)).

Approximately 300 millions of world population carry hepatitis B virus ("HBV") which may cause chronic infection, leading to cirrhosis and hepatocellular carcinoma (Tiollais P. and Buendia M.A., *Sci. Am.*, 264, 48(1991)). The HBV envelope consists of three proteins, major protein containing S antigen, middle protein containing S and pre-S2 antigens, and large protein containing S, pre-S2 and pre-S1 antigens (Neurath A.R. and Kent S.B., *Adv. Vir. Res.*, 34, 65-142(1988)). These surface antigens have been known to play important roles in the process of forming antibodies against HBV in hepatitis patient. The pre-S1 region, in particular, is found on infectious viral particles (Heermann et al., *J. Virol.*, 52, 396-402(1984)) and plays a role in attachment to cell surface infection (Neurath et al., *Cell*, 46, 429(1986); Pontisso et al., *Virol.*, 173, 533, (1989); Neurath et al., *Vaccine*, 7, 234(1989)). Thus a monoclonal antibody against the pre-S1 would be effective against viral infection.

The present inventors have previously reported a murine monoclonal antibody (KR127) against HBV pre-S1 (Korean Patent No. 246128), a murine monoclonal antibody KR127 gene encoding same (Korean Patent No. 250832) and a humanized antibody (HZKP127I) of KR127 prepared by CDR-grafting method (Korean Patent No. 246128).

The present inventors have further endeavored to develop a humanized antibody having minimized adverse immune response (HAMA response) as well as enhanced affinity to antigen, and found that HAMA response can be reduced when the amino acid residues of CDR of mouse antibody are replaced with those of human antibody.

Summary of the Invention

Accordingly, it is an object of the present invention to provide a process for preparing a humanized antibody which is expected to show lower HAMA response and has higher affinity than humanized antibody of the prior art.

It is another object of the present invention to provide a humanized antibody prepared according to said process.

It is a further another object of the present invention to provide a DNA encoding the heavy chain or light chain of said antibody and a vector comprising said DNA.

It is a still further object of the present invention to provide a

microorganism transformed with said vector.

In accordance with one aspect of the present invention, there is provided a process for preparing a humanized antibody comprising the steps of: (a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions; and (b) grafting the amino acid residues of said SDR to at least one of the corresponding amino acid sequences in human antibody variable regions.

10 **Brief Description of the Drawings**

The above and other objects and features of the present invention will become apparent from the following description of the invention taken in conjunction with the following accompanying drawings; which respectively show:

Fig. 1 : the procedure for constructing an expression vector of a chimeric heavy chain;

Fig. 2 : the nucleotide and amino acid sequence of the humanized heavy chain variable region (SEQ ID NOS: 27, 28, 31, 32, 35, 36);

Fig. 3 : the procedure for constructing an expression vector of a chimeric light chain;

Fig. 4 : the nucleotide and amino acid sequence of the humanized light chain variable region (SEQ ID NOS: 29, 30, 33, 34, 37, 38);

Fig. 5 : the affinity to antigen of a humanized antibody having a heavy chain CDR mutant;

Fig. 6 : the procedure for constructing an expression vector of the humanized antibody; and

Fig. 7: the affinity of the humanized antibody HuKR127 compared to the affinity of the antibody HuKR127I.

Detailed Description of the Invention

The humanized antibody of the present invention may be prepared by

grafting the amino acid residues of SDR of murine monoclonal antibody to the corresponding amino acid sequences in human antibody variable regions.

SDRs of the murine monoclonal antibody used in the present invention may be determined by independently replacing each amino acid residue of CDR of the murine monoclonal antibody with alanine, selecting
5 transformants which have lower affinity (K_D) to antigen than the original murine antibody and determining the replaced CDR amino acid residues of said transformants as SDRs.

Further, in order to enhance the affinity to antigen, the CDR residues
10 of a mouse antibody that increase the affinity and the framework residues that influence the conformation of CDR loops may also be grafted to the corresponding sites of human antibody.

For example, the present invention describes a process for preparing a humanized antibody for hepatitis B virus (HBV) pre-S1 by using murine
15 monoclonal antibody KR127 (Korean Patent No. 250832) as follows:

After selecting SDR amino acid residues, which play important roles in binding with antigen, from CDR of the murine monoclonal antibody KR127 heavy and light chains, chimeric heavy chain and chimeric light chain genes may be prepared by combining either the variable region of
20 KR127 antibody heavy chain with the constant region ($C_H 1$) of human antibody or the variable region of KR127 antibody light chain with the constant region (C_L) of human antibody.

SDRs of the murine monoclonal antibody for HBV pre-S1 are determined by replacing each amino acid residue of CDR HCDR1 (aa 31-35),
25 HCDR2 (aa 50-65) and HCDR3 (aa 95-102) of the heavy chain (SEQ ID NO: 2) and CDR LCDR1 (aa 24-34), LCDR2(aa 50-56) and LCDR3(aa 89-97) of the light chain (SEQ ID NO: 4) of the murine monoclonal antibody KR127 with alanine according to the alanine scanning mutagenesis method and selecting the amino acid residues (SDRs) whose replacement with
30 alanine bring about more than 3 times reduction in the affinity to antigen(K_D) as compared with the original murine antibody. Throughout this description, amino acid residue number is assigned according to Kabat numbering scheme (Kabat, E. A. et al, Sequences of Proteins of Immunological Interest. *National Institute of Health, Bethesda, MD.*, 1991).

35 Examples of preferred SDR include tryptophan at position 33 (it is represented as "Trp33"), Met34, and Asn35 of HCDR1; Arg50, Tyr52, and

Pro52a of HCDR2; Glu95, Tyr96, and Glu98 of HCDR3 of the murine monoclonal antibody KR127 heavy chain; Leu27b, Tyr27d, Ser27e; Asn28, Lys30, Tyr32, and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 of the murine monoclonal antibody KR127 light chain.

The humanized antibody of the present invention can be prepared by grafting one or more SDRs determined as above onto the human antibody heavy chain and/or light chain. The human antibody heavy chain which may be used in the present invention is human heavy chain DP7-JH4 consisting of human immunoglobulin germline VH gene segment DP7 (Tomlinson et al., *J. Mol. Biol.*, 227, 776-798, 1992) and JH4 segment (Ravetch et al., *Cell*, 27, 583-591, 1981). The human antibody light chain which may be used in the present invention is human light chain DPK12-JH4 consisting of human immunoglobulin germline VK gene segment DPK12 (Cox et al., *Eur. J. Immunol.*, 24, 827-836 (1994)) and JH4 segment (Hieter et al., *J. Biol. Chem.*, 257, 1516-1522 (1982)).

The humanized heavy chain of the present invention may be prepared by grafting at least one of Trp33, Met34, and Asn35 of HCDR1; Arg50, Tyr52, and Pro52a of HCDR2; Glu95, Tyr96, and Glu98 of HCDR3 of the murine monoclonal antibody KR127 heavy chain to the corresponding amino acid sequences in human antibody heavy chain. The inventive humanized light chain may be prepared by grafting at least one of Leu27b, Tyr27d, Ser27e; Asn28, Lys30, Tyr32, and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 of the murine monoclonal antibody KR127 light chain to the corresponding amino acid sequences in human antibody light chain DPH12-JK4.

Moreover, the affinity to antigen of the humanized antibody can be enhanced by the follow substitutions:

- (a) the amino acid residue at position 32 in HCDR1 of the modified human heavy chain DP7-JH4 by Ala;
- (b) the amino acid residue at position 97 in HCDR3 of the modified human heavy chain DP7-JH4 by Arg or Ala;
- (c) the amino acid residue at position 98 in HCDR3 of the modified human heavy chain DP7-JH4 by Val; and
- (d) the amino acid residue at position 102 in HCDR3 of the

modified human heavy chain DP7-JH4 by Arg or Ala.

In addition, Ala71 and Lys73 in framework region 3 in the heavy chain variable region of KR127, which affects the conformation of the CDR loop, may further be grafted to human heavy chain DP7-JH4. Also, Leu36 and Arg46 in framework region 2 in the light chain variable region of KR127, which affects conformation of CDR loop, may be further grafted to human light chain DPH12-JK4.

The heavy chain variable region of humanized antibody of the present invention has the amino acid sequence of SEQ ID NO: 2, preferably encoded by the nucleotide sequence of SEQ ID NO: 1 and the inventive light chain variable region of humanized antibody has the amino acid sequence of SEQ ID NO: 4, preferably encoded by the nucleotide sequence of SEQ ID NO: 3.

The humanized antibody heavy chain and light chain of the present invention may be encoded by a gene comprising a nucleotide sequence deduced from the humanized antibody heavy chain and light chain according to the genetic code. It is known that several different codons encoding a specific amino acid may exist due to the codon degeneracy, and, therefore, the present invention includes in its scope all nucleotide sequences deduced from the humanized antibody heavy chain and light chain amino acid sequence. Preferably, the humanized antibody heavy chain and light chain gene sequences include one or more preferred codons of host cell.

The humanized antibody consisted of the humanized heavy chain HuKR127HC of the present invention and humanized light chain HZKR127I prepared by CDR-grafting has an affinity to antigen of about over 50 times higher than that of the humanized antibody HZKR127I.

The humanized antibody consisting of the humanized heavy chain HuKR127KC of the present invention and humanized light chain HZKR127I prepared by CDR-grafting has an affinity to antigen equal to that of the humanized antibody HZKR127I.

The genes of humanized antibody heavy chain and light chain thus prepared may be inserted to pdCMV-dhfrC-HAV6 vector (KCTC 10028BP) to obtain an expression vector pdCMV-dhfrC-HuKR127 which can express both humanized antibody heavy chain HuKR127HC and light chain HZKR127I. The expression vector of the present invention may be introduced into microorganism, e.g., *E. coli* DH5a according to a

conventional transformation method to obtain transformants *E. coli* DH5α / pdCMV-dhfrC-HuKR127. The transformants *E. coli* DH5α / pdCMV-dhfrC-HuKR127 was deposited on March 13, 2002 with the Korean Collection for Type Cultures(KCTC)(Address: Korea Research Institute of Bioscience and Biotechnology(KRIBB), #52, Oun-dong, Yusong-ku, Taejon, 305-333, Republic of Korea) under the accession number, KCTC 10198BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

Meanwhile, CHO/HuKR127, CHO (Chinese hamster ovary) cell line transfected with vector pdCMV-dhfrC-HuKR127, was deposited on March 13, 2002 with the Korean Collection for Type Cultures(KCTC) under the accession number, KCTC 10199BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

The humanized antibody HuKR127 of the present invention produced by culturing the CHO/HuKR127 cell line has a higher affinity to antigen and is expected to reduce HAMA (human anti-mouse antibody) response to a greater extent than the conventional antibody prepared according to the CDR-grafting method.

Accordingly, the humanized antibody of the present invention can be used in preventing hepatitis B virus infection and treating chronic Hepatitis B.

Thus, for preventing hepatitis B virus infection and treating chronic Hepatitis B, a pharmaceutical formulation of the inventive humanized antibody may be prepared in accordance with any of the conventional procedures.

The pharmaceutical composition of the present invention can be administered via various routes including intravenous and intramuscular introduction. It should be understood that the amount of the active ingredient actually administered ought to be determined in light of various relevant factors including the condition to be treated, the chosen route of administration, the age, sex and body weight of the individual patient, and the severity of the patient's symptom; and, therefore, the above dose should not be intended to limit the scope of the invention in any way.

The following Examples are intended to further illustrate the present invention without limiting its scope.

Example 1: Preparation of mouse/human chimeric heavy chain gene

5 The gene encoding leader sequence and the $\gamma 1$ constant region of the human antibody heavy chain were separately prepared by carrying out PCR using pCMV-HKR127HC (Korean Patent No. 246128, KCTC 0531BP) as a template and a primer set of Ryu94 (SEQ ID NO: 5) and HUR43-1 (SEQ ID NO: 6) or HUR46-1 (SEQ ID NO: 9) and HUR31 (SEQ ID NO: 10).

10 The gene encoding heavy chain variable region of the murine monoclonal antibody KR127 was prepared by carrying out PCR using pKR127H (Korean Patent No. 250832, KCTC 0333BP) as a template and primers HUR44-1 (SEQ ID NO: 7) and HUR45-1 (SEQ ID NO: 8).

15 Ryu94: 5'-GAG AAT TCA CAT TCA CGA TGT ACT TG-3'
HUR43-1: 5'-CTG CTG CAG CTG GAC CTG ACT CTG GAC ACC ATT-3'
HUR44-1: 5'-CAG GTC CAG CTG CAG CAG TCT GGA CCT GAA CTG-3'
20 HUR45-1: 5'-TGG GCC CTT GGT GGA GGC TGC AGA GAC AGTGAC-3'
HUR46-1: 5'-GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG-3'
HUR31: 5'-CAG CGG CCG CTC ATT TAC CCG GGG ACA G-3'

25 Each PCR reaction was carried out using 10 ng of template, 1 μ l of each primer (50 ppm), 0.5 μ l of *Pfu* DNA polymerase (Promega), 4 μ l of 2.5 mM dNTPmix and 5 μ l of 10 x *Pfu* reaction buffer solution. After pre-denaturation at 95°C for 5 minutes, a PCR cycle was repeated 25 times, the
30 cycle being composed of: 95°C for 30 sec., 50°C for 30 sec. and 72°C for 45 sec. After annealing the DNA fragment obtained by using primers Ryu94 and HUR43-1, the DNA fragment obtained by using primers HUR44-1 and HUR45-1, and the DNA fragment obtained by using primers HUR46-1 and HUR31 were recombined by conducting recombinant PCR using
35 primers Ryu94 and HUR31. The recombinant PCR reaction was carried out using the same reaction buffer solution as used above. After pre-denaturation

at 95 °C for 5 minutes, a PCR cycle was repeated 30 times, the cycle being composed of: 95 °C for 30 sec., 50 °C for 30 sec. and 72 °C for 60 sec., and finally, the extension reaction was carried out at 72 °C for 5 min.

The chimeric heavy chain gene thus prepared was cleaved with
 5 *Eco*RI(GAATTC) and *Nde*I (GCGGCCGC) and inserted at the *Eco*RI/*Nde*I
 section of vector pcDdA (plasmid which is removed *Ap*I site in the multiple
 cloning site of pcDNA received from Invitrogen), to obtain vector
 pcDdAchKR127HC (Fig. 1). The base sequence of the chimeric heavy
 chain variable region gene (KR127VH) was confirmed by DNA sequence
 10 analysis (Fig. 2).

Example 2: Preparation of mouse/human chimeric light chain gene

The gene encoding reader sequence and the constant region of the
 15 human antibody light chain were each prepared by carrying out PCR using
 pKC-dhfr-HKR127 (Korean Patent No. 2000-33008, KCTC 0529BP) as a
 template and a primer set of Ryu86 (SEQ ID NO: 11) and HUR48 (SEQ ID
 NO: 12) or HUR51 (SEQ ID NO: 15) and CK1D (SEQ ID NO: 16).

The gene encoding light chain variable region of the murine
 20 monoclonal antibody KR127 was prepared by carrying out PCR using
 pKR127K (Korean Patent No. 250832, KCTC 0334BP) as a template and
 primers HUR49 (SEQ ID NO: 13) and HUR50 (SEQ ID NO: 14).

Ryu86: 5'-CAA AGC TTG GAA GCA AGA TGG ATT CA-3'
 25 HUR48: 5'-CAA GAT ATC CCC ACA GGT ACC AGA TAC-3'
 HUR49: 5'-TGT GGG GAT ATC TTG ATG ACC CAA ACT-3'
 HUR50: 5'-CAC AGA TCT TTT GAT TTC CAG CTT GGT-3'
 HUR51: 5'-ATC AAA AGA TCT GTG GCT GCA CCA TCT-3'
 CK1D: 5'-GCG CCG TCT AGA ATT AAC ACT CTC CCC TGT TGA
 30 AGC TCT TTG TGA CGG GCG AACTCAG-3'

Each PCR reaction was carried out according to the method described
 in Example 1 except that primers Ryu86 and CK1D were used to ligate the
 annealed DNA fragments obtained by PCR reactions.

35 The chimeric light chain gene thus prepared was cleaved with
*Hind*III (AAGCTT) and *Xba*I (TCTAGA) and inserted at the *Hind*III/*Xba*I

section of vector pBluescript KS, to obtain a recombinant plasmid. Subsequently, the recombinant plasmid was cleaved with *HindIII* and *ApaI* and inserted at the *HindIII/ApaI* section of vector pCMV-dhfr (KCTC 8671P), to obtain plasmid pKC-dhfr-chKR127(Fig. 3). The base sequence
 5 of the chimeric light chain variable region gene (KR127VK) was confirmed by DNA sequence analysis (Fig. 4).

Example 3: Mutation of CDR of chimeric KR127 antibody heavy chain by alanine injection

10

To examine whether each amino acid residue of KR127 heavy chain HCDR1 (aa 31-35), HCDR2(aa 50-65) and HCDR3 (aa 95-102) binds to antigen, PCR reaction was carried out using vector pcDdA-chKR127HC as a template to prepare a modified gene, wherein an amino acid residue of CDR
 15 was replaced with alanine (the replaced amino acid residue No. was indicated as Kabat number) (see Fig. 2).

A forward primer YM001N of SEQ ID NO: 17 was designed to provide the sequence corresponding to the reader sequence at the 5'-end of the chimeric heavy chain gene and *EcoRI* restriction site, and a reverse primer
 20 YM003 of SEQ ID NO: 18 was designed to have the sequence corresponding to the N-terminal downstream of CH1 domain of human heavy chain gene and *ApaI* restriction site.

YM001N: 5'-CCG GAA TTC ACA TTC ACG ATG TAC TTG-3'

25 YM003: 5'-TGC CCC CAG AGG TGC T-3'

The 5'-end primer ym257 of SEQ ID NO: 19 (corresponding to nucleotide Nos. 80 to 112 of SEQ ID NO: 1) was designed to replace Ser31 of HCDR1 with alanine (S31A) and the 3'-end primer YM258 of SEQ ID
 30 NO: 20 (corresponding to nucleotide Nos. 101 to 71 of SEQ ID NO: 1), to replace AGT (coding for Ser) of nucleotide Nos. 91 to 93 of HCDRI gene with GCT (coding for alanine).

Each PCR reaction was carried out according to the method described in Example 1 except that primer sets, YM001N and YM258; and ym258 and
 35 YM003, were used and also that primers YM001N and YM003 were used to recombine the annealed DNA fragments obtained by PCR.

The chimeric light chain gene thus prepared was cleaved with *EcoRI* and *ApaI* and inserted at the *EcoRI/ApaI* section of vector pcDdA-chKR127HC prepared in Example 1, to obtain pcDdA-chKR127HC-S31A. The base sequence of the humanized antibody heavy chain variable region
5 gene was confirmed by DNA sequence analysis. Vectors containing mutants thus prepared are shown in Table 1.

In Table 1, primer and mutation positions are numbered based on the base sequence of SEQ ID NO: 1.

Table 1

CDR	primer		primer position	mutation position	mutant	vector
HCDR1	F	ym257	80-112	91-93	Ser(AGT)→Ala(GCT)	pcDdA-chKR127HC-S31A
	R	YM258	101-71			
	F	ym259	83-112	94-96	Ser(TCT)→Ala(GCT)	pcDdA-chKR127HC-S32A
	R	YM260	106-73			
	F	ym261	86-117	97-99	Trp(TGG)→Ala(GCG)	pcDdA-chKR127HC-W33A
	R	YM262	108-76			
	F	ym263	90-118	100-102	Met(ATG)→Ala(GCG)	pcDdA-chKR127HC-M33A
	R	YM264	111-79			
	F	ym265	94-120	103-105	Asn(AAC)→Ala(GCC)	pcDdA-chKR127HC-N35A
	R	ym266	112-81			
HCDR2	F	YM221	139-174	148-150	Arg(CGG)→Ala(GCC)	pcDdA-chKR127HC-R50A
	R	YM222	158-128			
	F	YM225	143-178	151-153	Ile(ATT)→Ala(GCT)	pcDdA-chKR127HC-I51A
	R	YM226	162-131			
	F	YM227	145-180	154-156	Tyr(TAT)→Ala(GCT)	pcDdA-chKR127HC-Y52A
	R	YM228	165-135			
	F	ym229	148-181	157-159	Pro(CCT)→Ala(GCT)	pcDdA-chKR127HC-P52aA
	R	YM230	167-136			
	F	ym231	150-186	160-162	Gly(GGA)→Ala(GCA)	pcDdA-chKR127HC-G53A
	R	YM232	173-145			
	F	ym233	152-188	163-165	Asp(GAT)→Ala(GCT)	pcDdA-chKR127HC-D54A
	R	YM234	176-144			
	F	ym235	155-193	166-168	Gly(GGA)→Ala(GCA)	pcDdA-chKR127HC-G55A
	R	YM236	178-146			
	F	ym237	158-194	169-171	Asp(GAT)→Ala(GCT)	pcDdA-chKR127HC-D56A
	R	ym238	184-149			
	F	ym239	160-195	172-174	Thr(ACT)→Ala(GCT)	pcDdA-chKR127HC-T57A
	R	ym240	185-150			
	F	ym241	164-196	175-177	Asn(AAC)→Ala(GCC)	pcDdA-chKR127HC-N58A
	R	ym242	187-150			
HCDR3	F	YM207	286-317	295-297	Glu(GAG)→Ala(GCG)	pcDdA-chKR127HC-E95A
	R	YM208	305-274			
	F	YM209	289-316	298-300	Tyr(TAC)→Ala(GCC)	pcDdA-chKR127HC-Y96A
	R	YM210	307-276			
	F	YM211	292-318	301-303	Asp(GAC)→Ala(GCC)	pcDdA-chKR127HC-D97A
	R	YM212	313-279			
	F	YM213	296-321	304-306	Glu(GAG)→Ala(GCG)	pcDdA-chKR127HC-E98A
	R	YM214	315-285			
	F	YM255	303-327	310-312	Tyr(TAC)→Ala(GGC)	pcDdA-chKR127HC-Y102A
	R	YM256	319-289			

Test Example 1: Expression of chimeric antibody having a modified heavy chain and its affinity to antigen

(step 1) Expression of chimeric antibody

5 COS7 cells (ATCC CRL-1651) were seeded to DMEM media (GIBCO) containing 10% bovine serum and subcultured in an incubator at 37°C under an atmosphere of 5% CO₂. 1 x 10⁶ cells thus obtained were seeded to the same media and incubated at 37°C overnight. Thus, 5 µg of plasmid pKC-dhfr-chKR127 (expressing chimeric light chain) obtained in
10 Example 2, 5 µg of plasmid obtained in Example 3 were diluted with OPTI-MEM I (GIBCO) to 800 µl. 50 µl of Lipofectamine (GIBCO) were diluted with the same solution to 800 µl. The resulting solutions were added to a 15 ml tube, mixed and then, kept at room temperature for more than 15 minutes. Meanwhile, COS7 cells incubated as above were washed three times with
15 OPTI-MEM I. Then, 6.4 ml of OPTI-MEM I was added to the DNA-Lipofectamine mixture and the resulting solution was evenly distributed on the COS7 cells, which were cultured for 48 hours in a 5% CO₂ incubator to obtain a supernatant. The resulting solution was subjected to sandwich ELISA analysis using anti-human IgG (Sigma) as a capture antibody and anti-human
20 antigen (Fc-specific)-horseradish peroxidase (PIERCE) as a secondary antibody to confirm the expression of the chimeric antibody.

(step 2) Affinity to antigen

25 150 ng of HBV recombinant antigen GST-pre-S1(1-56) (H. S. Kim and H. J. Hong, *Biotechnology Letters*, 17, 871-876(1995)) was coated to each well of a microplate and 5 ng of the supernatant obtained in Step 1 was added to each well. The resulting solution was subjected to indirect ELISA using the same secondary antibody as used in step 1, followed by measuring the absorbance at 450 nm. Further, the affinity to antigen (K_D) of each
30 modified heavy chain was determined by competitive ELISA method (Ryu et al., *J. Med. Virol.*, 52, 226(1997)) and compared with that of pCK-dhfr-chKR127 containing wildtype chimeric heavy chain. The result is shown in Table 2.

Table 2

CDR	Mutant	K_D (nM)
	WT	11.0 ± 1.664
H1	S31A	14.67 ± 2.386
	S32A	8.455 ± 0.840
	W33A	>10000
	M34A	>10000
	N35A	>10000
H2	R50A	>10000
	I51A	12.8 ± 1.05
	Y52A	276.8 ± 23.60
	P52aA	170.3 ± 5.318
	G53A	7.697 ± 0.980
	D54A	1.663 ± 0.477
	G55A	5.766 ± 0.211
	D56A	6.59 ± 1.09
	T57A	13.68 ± 4.016
	N58A	1.568 ± 0.085
H3	E95A	>10000
	Y96A	>10000
	D97A	0.57 ± 0.03
	E98A	64.2 ± 7.78
	Y102A	3.581 ± 0.457

As shown in Table 2, the affinities to antigen of the mutants obtained by replacing Trp33, Met34, or Asn35 of HCDR1; Arg50, Tyr52, or Pro52a of HCDR2; Glu95, Tyr96, or Glu98 of HCDR3 with alanine were more than 3 times lower than that of wild type. However, a mutant having alanine substituting for Asp97 or Tyr102 residue of HCDR3 exhibited an enhanced affinity to antigen.

Example 4: Preparation of HCDR3 mutants and their affinities to antigen

(step 1) D97R and E98V mutants

Each mutant was prepared by replacing Asp97 or Glu98 of HCDR3 with arginine as a positively charged amino acid (it is represented as "D97R") or valine as a neutral amino acid (it is represented as "E98V") according to the site-directed mutagenesis as used in Example 3. Vectors containing mutants prepared as above are shown in Table 3.

Table 3

CDR	primer		primer position	mutation position	mutant	vector
HCDR3	R	P1	312-279	301-303	Asp(GAC)→	pcDdA-chKR127HC-D97R
	F	P2	295-326		Arg(CGG)	
	R	P3	312-279	301-303	Asp(GAC)→	pcDdA-chKR127HC-D97V
	F	P4	295-326		Val(GTT)	
	R	P5	312-279	304-306	Glu(GAG)→	pcDdA-chKR127HC-E98R
	F	P6	295-326		Arg(CGG)	
	R	P7	312-279	304-306	Glu(GAG)→	pcDdA-chKR127HC-E98V
	F	P8	295-326		Val(GTT)	

Then, each mutant thus obtained was measured for its affinity to antigen in according to the method described in Test Example 1 and compared with that of the wild type.

As shown in Fig 5, the affinity to antigen of D97R was more than 3 times higher than that of the wild type, which the affinity to antigen of E98V, more than 4 times higher than that of the wild type. However, mutant E98R showed a low affinity to antigen.

(Step 2) D97R/E98V mutant

To prepare D97R/E98V mutant containing both D97R and E98V, which were found to be mutants having high affinity to antigen, PCR reaction was carried out using pcDdA-chKR127HC-D97R which contains D97R gene as a template and primers P7 and P8.

Then, the D97R/E98V mutant thus obtained was measured for its affinity to antigen in according to the method described in Test Example 1.

As shown in Fig 5, the affinity to antigen of D97R/E98V was more than 15 times higher than that of the wild type.

(Step 3) D97R/E98V/Y102A mutant

To prepare D97R/E98V/Y102A mutant containing D97R, E98V and Y102A, PCR reaction was carried out using pcDdA-chKR127HC-RV containing D97R/E98V as a template and primers YM255 and YM256.

Then, the D97R/E98V/Y102A mutant (hereinafter "RVAA") thus

obtained was measured for its affinity to antigen in according to the method described in Test Example 1.

As shown in Fig 5, the affinity to antigen of D97R/E98V/Y102A was similar to that of D97R/E98V.

5

(Step 4) D97R/E98V/Y102E and D97R/E98V/Y102R mutants

To prepare D97R/E98V/Y102E mutant and D97R/E98V/Y102R mutant, PCR reaction was carried out using pcDdA-chKR127HC-RV containing D97R/E98V as a template, and primer sets P17/P18 and P19/P20, respectively.

10

Vector containing mutants prepared above are shown in Table 4.

Table 4

15

	primer		primer position	mutation position	mutant	vector
HCDR3	R	P17	312-279	307-309	Tyr (TAC)→	pcDdA-chKR127HC-RVAE
	F	P18	295-326		Glu(GAG)	
	R	P19	312-279	307-309	Tyr (TAC)→	pcDdA-chKR127HC-RVAR
	F	P20	295-326		Arg(CGT)	

20

Then, D97R/E98V/Y102E mutant (hereinafter "RVAE") and D97R/E98V/Y102R mutant (hereinafter "RVAR") thus obtained were measured for respective affinities to antigen in according to the method described in Test Example 1.

25

As shown in Fig 5, the affinity to antigen of RVAE was similar to that of RVAA, while the affinity to antigen of RVAR was higher than that of RVAA.

30 Test Example 2: Measurement of affinity to antigen of RVAR

The RVAR mutant prepared in step 4 of Example 4 was subjected to competitive ELISA to measure its affinity to antigen as follows:

COS7 cells were transfected with the plasmid prepared in step 4 of Example 4 and the plasmid expressing chimeric light chain(pKC-dhfr-chKR127) prepared in Example 2 to produce an antibody. 5 ng of the antibody thus obtained was reacted with pre-S1 antigen (1×10^{-7} to 1×10^{-12}

35

M) at 37 °C for 2 hours. The resulting solution was added to each well of a 96-well microplate coated with pre-S1 antigen and reacted at 37°C for 30 minutes, and then the resulting solution was subjected to ELISA analysis according to the method described in Example 4. Used as a control is
 5 chimeric antibody (chKR127) obtained from COS7 cells transfected with pcDdA-chKR127HC and pKC-dhfr-chKR127.

The affinity to antigen of RVAR was about 1.8×10^{-10} M, which is 45 times higher than that of chKR127, about 8.2×10^{-9} M

10 Example 5: Mutation of CDR of chimeric KR127 antibody light chain by alanine injection

To examine the affinity of each amino acid residue of KR127 light chain LCDR1 (aa 24-34), LCDR2(aa 50-60) and LCDR3 (aa 89-97) to antigen, PCR reaction was carried out using vector pKC-dhfr-chKR127 as a
 15 template to prepare a modified gene having each amino acid residue of CDR replaced with alanine (the replaced amino acid residue Number was indicated as Kabat number)(see Fig. 2).

Forward primer YM004 of SEQ ID NO: 21 was designed to provide the sequence corresponding to the reader sequence at the 5'-end of the
 20 chimeric light chain gene and the *HindIII* restriction site, and a reverse primer YM009 of SEQ ID NO: 22 was designed to have the sequence corresponding to the N-terminal region of human light chain gene and the *BsiWI*(CGTACG) restriction site. These primers were used in preparation of mutants of light chain CDR residue.

25

YM004: 5'-CCA AAG CTT GGA AAG ATG GAT TCA CAG-3'

YM009: 5'-GCA GCC ACC GTA CGT TTG ATT TCC ACC TTG GT-3'

Forward primer YM135 was designed to replace Ser26 of LCDR1
 30 with alanine (S26A) and a reverse primer YM136, to replace AGT coding for Ser at the nucleotide Nos. 76 to 78 of LCDRI gene with GCT coding for alanine.

PCR reactions were carried out according to the method described in Example 1 except that primer sets, YM004/YM135, and YM136/YM009, were
 35 used and that primers YM004 and YM009 were used to recombine the annealed DNA fragments obtained by PCR.

The variable region gene of the mutant thus prepared was cleaved with *Hind*III and *Bsi*WI and inserted at the *Hind*III/*Bsi*WI section of vector pKC-dhfr-chKR127, to obtain pKC-dhfr-chKR127BS-S26A. The base sequence of the modified chimeric light chain variable region gene was confirmed by DNA sequence analysis. The vectors containing mutants prepared above are shown in Table 5.

In Table 5, the primer and mutation positions are numbered based on the base sequence of SEQ ID NO: 3.

Table 5

10

15

20

25

30

35

	primer		primer position	mutation position	mutant	vector
15	F	YM135	67-102	76-78	Ser(AGT)-Ala(GCT)	pKC-dhfr-chKR127BS-S26A
	R	YM136	86-54			
	F	YM137	69-107	79-81	Gln(CAG)-Ala(GCG)	pKC-dhfr-chKR127BS-Q27A
	R	YM138	91-56			
	F	YM139	70-111	82-84	Ser(AGC)-Ala(GCC)	pKC-dhfr-chKR127BS-S27aA
	R	YM140	94-58			
20	F	YM141	73-114	85-87	Leu(CTC)-Ala(GCC)	pKC-dhfr-chKR127BS-L27bA
	R	YM142	98-64			
	F	YM143	73-116	88-91	Leu(TTA)-Ala(GCA)	pKC-dhfr-chKR127BS-L27cA
	R	YM144	102-68			
	F	YM145	79-118	91-93	Tyr(TAT)-Ala(GCT)	pKC-dhfr-chKR127BS-Y27dA
	R	YM146	103-69			
25	F	YM147	83-119	94-96	Ser(AGT)-Ala(GCT)	pKC-dhfr-chKR127BS-S27eA
	R	YM148	107-69			
	F	YM149	84-120	97-99	Asn(AAT)-Ala(GCT)	pKC-dhfr-chKR127BS-N28A
	R	YM150	110-70			
	F	YM151	88-127	100-102	Gly(GGA)-Ala(GCA)	pKC-dhfr-chKR127BS-G29A
	R	YM152	114-74			
30	F	YM153	91-130	103-105	Lys(AAA)-Ala(GCA)	pKC-dhfr-chKR127BS-K30A
	R	YM154	116-77			
	F	YM155	93-132	106-108	Thr(ACC)-Ala(GCC)	pKC-dhfr-chKR127BS-T31A
	R	YM156	118-80			
	F	YM103	99-133	109-111	Tyr(TAT)-Ala(GCT)	pKC-dhfr-chKR127BS-Y32A
	R	YM104	120-83			
35	F	N34A-F	106-132	115-118	Asn(AAT)-Ala(GCT)	pKC-dhfr-chKR127BS-Y34A
	R	N34A-R	126-100			

		primer	primer position	mutation position	mutant	vector
5	LCDR2	F	YM129	151-188	Leu(CTG)- Ala(GCG)	pKC-dhfr-chKR127BS-L50A
		R	YM130	175-140		
		F	YM131	153-191	Val(GTG)- Ala(GCG)	pKC-dhfr-chKR127BS-V51A
		R	YM132	179-145		
		F	YM133	157-192	Ser(TCT)- Ala(GCT)	pKC-dhfr-chKR127BS-S52A
		R	YM134	181-147		
		F	K53A-F	163-187	Lys(AAA)- Ala(GCA)	pKC-dhfr-chKR127BS-K53A
		R	K53A-R	178-154		
		F	L54A-F	163-189	Leu(CTG)- Ala(GCG)	pKC-dhfr-chKR127BS-L54A
		R	L54A-R	180-159		
		F	D55A-F	170-195	Asp(GAC)- Ala(GCC)	pKC-dhfr-chKR127BS-D55A
		R	D55A-R	184-163		
		F	K56A-F	175-198	Ser(TCT)- Ala(GCT)	pKC-dhfr-chKR127BS-S56A
		R	K56A-R	190-168		
15	LCDR3	F	YM113	270-304	Val(GTG)- Ala(GCG)	pKC-dhfr-chKR127BS-V89A
		R	YM114	292-258		
		F	YM115	274-307	Gln(CAA)- Ala(GCA)	pKC-dhfr-chKR127BS-Q90A
		R	YM116	294-259		
		F	YM117	277-310	Gly(GGT)- Ala(GCT)	pKC-dhfr-chKR127BS-G91A
		R	YM118	296-265		
		F	YM119	281-310	Thr(ACA)- Ala(GCA)	pKC-dhfr-chKR127BS-T92A
		R	YM120	302-266		
		F	YM121	282-313	His(CAT)- Ala(GCT)	pKC-dhfr-chKR127BS-H93A
		R	YM122	304-271		
		F	YM111	286-314	Phe(TTT)- Ala(GCT)	pKC-dhfr-chKR127BS-F94A
		R	YM112	307-274		
		F	YM123	286-317	Pro(CCT)- Ala(GCT)	pKC-dhfr-chKR127BS-P95A
		R	YM124	308-278		
		F	YM125	292-319	Gln(CAG)- Ala(GCG)	pKC-dhfr-chKR127BS-Q96A
		R	YM126	311-279		
		F	YM127	294-320	Thr(ACG)- Ala(GCG)	pKC-dhfr-chKR127BS-T97A
		R	YM128	313-282		

Test Example 3: Measurement of affinity to antigen of light chain mutant

COS7 cell was transfected with each of the light chain mutants prepared in Example 5 and the plasmid expressing chimeric heavy chain(pcDdA-chKR127HC) to produce an antibody. The antibody obtained

was measured for its affinity to antigen in accordance with the method described in Test Example 1.

Table 6 shows the results obtained for the mutants and pdDA-chKR127HC containing wildtype chimeric KR127 heavy chain.

Table 6

CDR	mutant	K_D (nM)
L1	S26A	6.49 ± 0.244
	Q27A	14.2 ± 2.29
	S27aA	37.9 ± 6.66
	L27bA	>10000
	L27cA	36.8 ± 11.01
	Y27dA	1032.7 ± 56.1
	S27eA	>10000
	N28A	>10000
	G29A	23.94 ± 2.62
	K30A	>10000
	T31A	13.19 ± 1.98
	Y32A	>10000
	N34A	>10000
L2	L50A	159.4 ± 21.37
	V51A	37.00 ± 10.33
	S52A	14.08 ± 0.509
	K53A	7.928 ± 0.976
	L54A	12.57 ± 2.453
	D55A	225.2 ± 2.970
	S56A	12.95 ± 0.367
L3	V89A	121.2 ± 4.62
	Q90A	>10000
	G91A	>10000
	T92A	74.2 ± 2.90
	H93A	54.5 ± 4.48
	F94A	>10000
	P95A	>10000
	Q96A	293.6 ± 7.13
	T97A	17.3 ± 2.56

As shown in Table 6, the affinities to antigen of the mutants obtained by replacing the Leu27b, Tyr27d, Ser27e, Asn28, Lys30, Tyr32, and Asn34 of LCDR1; Leu50 and Asp55 of LCDR2; and Val89, Gln90, Gly91, Thr92, His93, Phe94, Pro95, and Gln96 of LCDR3 with alanine, respectively, were more than 3 times lower than that of the wild type. Therefore, these residues was determined as SDR.

Example 6: Preparation of humanized heavy chain by SDR-grafting method

A humanized heavy chain was prepared using DP7-JH4, a human heavy chain constructed by combining human immunoglobulin germline VH gene segment DP7 (Tomlinson et al., *J. Mol. Biol.*, 227, 776-798, 1992) having an amino acid sequence similar to KR127 heavy chain variable regions and human immunoglobulin germline JH4 segment (Ravetch et al., *Cell*, 27, 583-591 (1981)).

The Trp33 and Asn35 in HCDR1 of the KR127 were grafted into the DP7-JH4. The Met34 in HCDR1 of the KR127 is identical to that of DP7-JH4. Further, to inhibit lowering the affinity to antigen, Tyr32 in HCDR1 of the KR127 was replaced with alanine of HCDR1 of a human antibody (Gen Bank data base 75023 (SAWMN)).

The Arg50 and Tyr52 in HCDR2 of the KR127 were grafted onto the DP7-JH4. The Pro52a in HCDR2 of the KR127 is identical to that of DP7-JH4.

The Asp95, Tyr96, Arg97, Val98, and Arg102 of HCDR3 were grafted into DP7-JH4.

Further, Ala71 and Lys73 of FR 3 (framework region 3) in the heavy chain variable region of KR127 antibody which affects the conformation of CDR loops were grafted thereto.

Then, PCR reaction was carried out using primers Ryu166 of SEQ ID NO: 23 and Hur37 of SEQ ID NO: 24 according to the method described in Example 3 to obtain a humanized heavy chain variable region gene, HuKR127VH-VII.

Ryu 166: 5'-GGA TTT GTC TGC AGT CAT TGT GGC TCT GCC CTG GAA CTT-3'

Hur 37: 5'-GAC AAA TCC ACG AGC ACA GTC TAC ATG-3'

The base sequence of the humanized heavy chain variable region gene was determined by DNA sequence analysis (Fig. 2). Then, the gene was cleaved with *EcoRI* and *ApaI* and inserted at the *EcoRI/ApaI* section of vector pDdA-chKR127HC to obtain pHuKR127HC.

A humanized antibody was prepared by combining humanized heavy chain thus obtained and the humanized antibody HZKR127I light chain described in Korean Patent No. 246128 and measured the affinity to antigen

was numbered according to the method described in Test Example 2. Humanized antibody HZKR127I was used as a control.

The affinity to antigen of the humanized antibody of about 1.5×10^{-10} M was about 50 times higher than that of HZKR127I, about 8.2×10^{-9} M.

5

Example 7: Preparation of humanized light chain by SDR-grafting method

A humanized light chain was prepared using DP7-JH4, a human light chain constructed by combining human immunoglobulin germline VK gene segment DPK12 (Cox et al., *Eur. J. Immunol.*, 24, 827-836 (1994)) having an amino acid sequence similar to KR127 light chain variable regions and human immunoglobulin germline JK4 segment (Hieter et al., *J. Biol. Chem.*, 257, 1516-1522 (1982)).

The Tyr27d, Asn28 and Asn34 in LCDR1 of KR127 were grafted into the DPK12-JK4. The amino acid residues at position 27b, 27e, 30 and 32 of DP7 is identical to those of KR127 light chain.

The Leu50 and Asp55 in LCDR2 of KR127 were grafted into the DPK12-JK4 gene.

The Val89, Gly91, Thr92, His93, Phe94, and Gln96 in LCDR3 of KR127 were grafted into the DPK12-JK4. The residues at positions 90 and 95 of DP7 is identical to those of KR127.

Further, Leu36 and Arg46 of FR 2 in the light chain variable region of KR127 antibody (which acts on interaction with heavy chain or CDR) were grafted thereto.

Then, PCR reaction was carried out using primers Ryu118 of SEQ ID NO: 25 and Ryu119 of SEQ ID NO: 26 according to the method described in Example 3 to prepare a humanized light chain variable region gene, HuKR127VH-IV.

Ryu 118: 5'-CTG TGG AGG CTG GCC TGG CTT CTG TAA TAA CCA-3'
Ryu 119: 5'-GGC CAG CCT CCA CAG CTC CTA ATC TAT CTG-3'

The base sequence of the humanized light chain variable region gene was determined by DNA sequence analysis (see HZIV of Fig. 4). Then, the gene was cleaved with *Hind*III and *Bsi*WI and inserted at the *Hind*III/*Bsi*WI section of vector pKC-dhfr-chKR127BS to obtain pHuKR127KC.

A humanized antibody was prepared by combining humanized light

chain thus obtained and the humanized antibody HZKR127I heavy chain described in Korean Patent No. 246128 and its affinity to antigen was measured according to the method described in Test Example 2. Humanized antibody HZKR127I was used as a control.

- 5 The affinities to antigen of the humanized antibody of about 8.4×10^{-9} M was similar to that of HZKR127I, about 8.2×10^{-9} M.

Example 8: Preparation of humanized antibody and measurement of the affinity to antigen

- 10 To prepare a plasmid containing humanized heavy chain plasmid pHuKR127HC and humanized light chain plasmid pHuKR127KC, the *EcoRI/ApaI* fragment containing humanized heavy chain variable region gene of pHuKR127HC and the *HindIII/BsiWI* fragment containing humanized light chain variable region gene of pHuKR127KC were inserted
15 at the *EcoRI/ApaI* and *HindIII/BsiWI* sections of vector pdCMV-dhfrC-HAV6 (KCTC 10028BP), respectively, to obtain plasmid pdCMV-dhfrC-HuKR127 (Fig. 6). *E. coli* DH5 α was transformed with the plasmid thus obtained and the transformed *E. coli* DH5 α /pdCMV-dhfrC-HuKR127 was deposited on March 13, 2002 with the Korean Collection for Type
20 Cultures(KCTC)(Address: Korea Research Institute of Bioscience and Biotechnology(KRIBB), #52, Oun-dong, Yusong-ku, Taejon, 305-333, Republic of Korea) under the accession number, KCTC 10198BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent
25 Procedure.

 To prepare cell line expressing the humanized antibody, dhfr-defected CHO (chinese hamster ovary) cells were transformed with plasmid pdCMV-dhfrC-HuKR127 as follows:

- 30 CHO cells (ATCC CRL 9096) were seeded to DMEM/F12 media (GIBCO) containing 10% fetal bovine serum and subcultured in an incubator at 37°C under an atmosphere of 5% CO₂. 5×10^5 cells thus obtained were seeded to the same media and incubated at 37°C overnight, followed by washing 3 times with OPTI-MEMI solution (GIBCO).

- 35 Meanwhile, 5 μ g of the plasmid pdCMV-dhfrC-HuKR127 was diluted in 500 μ l of OPTI-MEMI solution. 25 μ l of Lipofectamine was diluted in 500 μ l of the same solution. The resulting solutions were added

to a 15 ml tube, mixed, and then, kept at room temperature for more than 15 minutes. Then, 2 ml of OPTI-MEM I was added to by DNA-Lipofectamine mixture and the resulting solution was distributed evenly on the COS7 cells to be kept in a 5% CO₂ incubator at 37°C for 6 hours. Added thereto was 3
5 ml of DMEM/F12 containing 20% fetal bovine serum and cultured for 48 hours.

Then, CHO cells were taken up with trypsin and cultured in a-MEM media(GIBCO) of 10 % dialyzed fetal bovine serum containing G418 (GIBCO BRL, 550 mg/l) for 2 weeks. After confirming of antibody-producing
10 ability of the transformed clone, the clone was cultured in a-MEM media of 10 % dialyzed fetal bovine serum containing 20nM MTX to induce amplification of gene.

Cell line CHO/HuKR127 having the highest antibody-productivity was selected from the clones and deposited on March 13, 2002 with the Korean
15 Collection for Type Cultures(KCTC) under the accession number, KCTC 10199BP, in accordance with the terms of Budapest Treaty on the International Recognition of the Deposit of Microorganism for the Purpose of Patent Procedure.

To measure the affinity to antigen of the humanized antibody
20 HuKR127, CHO cell line thus obtained was mass cultured in a serum-absence media (CHO-SFMII, GIBCO) and subjected to protein G-shepharose 4B column (Pharmacia). Then, the antibody absorbed on the column was eluted with 0.1 M glycine solution (pH 2.7) and neutralized with 1.0 M tris solution (pH 9.0), followed by dialyzing in PBS buffer (pH 7.0). Further, the
25 affinity to antigen of the purified antibody was determined by the competitive ELISA method described in Test Example 2 and compared with that of a control, humanized HuKR127I. The result was shown in Fig. 7.

As shown in Fig. 7, the affinity to antigen of the humanized antibody of the present invention of 1.6×10^{-10} M was about 50 times higher than 8.2×10^{-9} M3 of the control group.
30

Example 9: Confirmation of immune-response induction of humanized antibody

To confirm whether the humanized antibody of the present invention
35 (HuKR127) prevents HAMA response, an analysis was conducted according to the TEPITOPE method (Sturniolo et al., *Nature Biotechnology*, 17, 555-

561, 1999) to examine whether a peptide sequences which can bind to MHC (major histocompatibility complex) class II exists in the heavy and light chain variable regions of the humanized antibody.

5 Tables. 7 and 8 show the results of such analysis for MHC class II-binding peptide sequences in the heavy chain variable regions of HuKR127 and the light chain variable regions of HuKR127, respectively.

Table 7

antibody	HzKR127I		HuKR127	
	peptide	MHC class II	peptide	MHC class II
MHC class II -binding	LVQSGAEVV	DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0311 DRB1_0421 DRB1_0701 DRB1_0703	LVQSGAEVK	0
	VKPGASVKV	DRB1_0102	KKPGASVKV	0
	FSSSWMNWV	DRB1_0703	FTSAWMNVV	0
	WIGRIYPGD	DRB1_0801 DRB1_0817	WMGRIYPSG	0
	FQ GKATLTA	DRB1_0401 DRB1_0402 DRB1_0405 DRB1_0408 DRB1_0421 DRB1_0426 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1114 DRB1_1120 DRB1_1121 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323	FQGRVTMTA	DRB1_0305 DRB1_0401 DRB1_0402 DRB1_0408 DRB1_0426 DRB1_0801 DRB1_0802 DRB1_0804 DRB1_0806 DRB1_0813 DRB1_0817 DRB1_1101 DRB1_1114 DRB1_1120 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1321 DRB1_1323 DRB1_1502
	YWGQGLVT	DRB1_0401 DRB1_0405 DRB1_0421 DRB1_0426	RWGQGLVT	0
	IGRIYPGDG	DRB5_0101 DRB5_0105	MGRYIPSGG	DRB1_0404 DRB1_0405 DRB1_0410 DRB1_0423
	YAQKFQGKA	DRB1_0802	YAQKFQGRV	0
	VYFCAREYD	DRB1_1304	VYYCAREYR	DRB1_0301
	YWGQGLVT	DRB1_0401 DRB1_0405 DRB1_0421 DRB1_0426	RWGQGLVT	0
total		50		26

Table 8a

antibody	H2K127I		HuK127	
	peptide	MHC class II	peptide	MHC class II
MHC class II -binding	ILMTQTPLS	DRB1_0301 DRB1_0305 DRB1_0306 DRB1_0307 DRB1_0308 DRB1_0309 DRB1_0311 DRB1_0401 DRB1_0402 DRB1_0404 DRB1_0405 DRB1_0408 DRB1_0410 DRB1_0421 DRB1_0423 DRB1_0426 DRB1_0804 DRB1_1101 DRB1_1102 DRB1_1104 DRB1_1106 DRB1_1107 DRB1_1114 DRB1_1121 DRB1_1128 DRB1_1301 DRB1_1304 DRB1_1305 DRB1_1307 DRB1_1311 DRB1_1321 DRB1_1322 DRB1_1323 DRB1_1327 DRB1_1328	IVMTQTPLS	0
	LMTQTPLSL	DRB1_0101 DRB1_0102 DRB1_1304	VMTQTPLSL	0
	WLLQKPGQS	DRB1_0101 DRB1_0305 DRB1_0309 DRB1_0401 DRB1_0408 DRB1_0421 DRB1_0426 DRB1_0802 DRB1_1101 DRB1_1107 DRB1_1114 DRB1_1120 DRB1_1128 DRB1_1302 DRB1_1305 DRB1_1307 DRB1_1321 DRB1_1323 DRB5_0101 DRB1_0105	WLLQKPGQP	0
	YYCVQGTHF	DRB1_0101 DRB1_0701 DRB1_0703 DRB5_0101 DRB5_0105	YYCVQGTHF	DRB1_0101 DRB1_0701 DRB1_0703 DRB5_0101 DRB5_0105
	YCVQGTHFP	DRB1_0401 DRB1_0421 DRB1_0426	YCVQGTHFP	DRB1_0401 DRB1_0421 DRB1_0426

Table 8b

antiboby		HzKR127I		HuKR127					
5		peptide	MHC class II	peptide	MHC class II				
		VGYYVCVQG	DRB1_0806	VGYYVCVQG	DRB1_0806				
		IYLVSKLDS	DRB1_0301	IYLVSNRDS	DRB1_0402				
			DRB1_0305		DRB1_0404				
			DRB1_0306		DRB1_0405				
			DRB1_0307		DRB1_0408				
			DRB1_0308		DRB1_0410				
			DRB1_0309		DRB1_0423				
DRB1_0311	DRB1_0804								
DRB1_0405	DRB1_1102								
DRB1_0410	DRB1_1104								
DRB1_0801	DRB1_1106								
DRB1_0802	DRB1_1114								
DRB1_0804	DRB1_1121								
DRB1_0806	DRB1_1301								
DRB1_0813	DRB1_1307								
DRB1_0817	DRB1_1311								
DRB1_1101	DRB1_1322								
DRB1_1102	DRB1_1323								
DRB1_1104	DRB1_1327								
DRB1_1106	DRB1_1328								
DRB1_1107	DRB5_0101								
DRB1_1114	DRB5_0105								
DRB1_1120									
DRB1_1121									
DRB1_1128									
DRB1_1301									
DRB1_1302									
DRB1_1304									
DRB1_1305									
DRB1_1307									
DRB1_1311									
DRB1_1321									
DRB1_1322									
DRB1_1323									
DRB1_1327									
DRB1_1328									
DRB1_1501									
DRB1_1506									
10									
						LIYLVSKLD	DRB1_0806	LIYLVSNRD	DRB1_0401
							DRB1_1304		DRB1_0404
DRB1_1321	DRB1_0405								
15									
						YLVSKLDSG	0	YLVSNRDSG	DRB1_0408
									DRB1_0410
	DRB1_0421								
20									
									DRB1_0423
									DRB1_0426
	DRB1_1304								
25									
30									
total	106		40						

While the embodiments of the subject invention have been described and illustrated, it is obvious that various changes and modifications can be made therein without departing from the spirit of the present invention which
5 should be limited only by the scope of the appended claims.

30

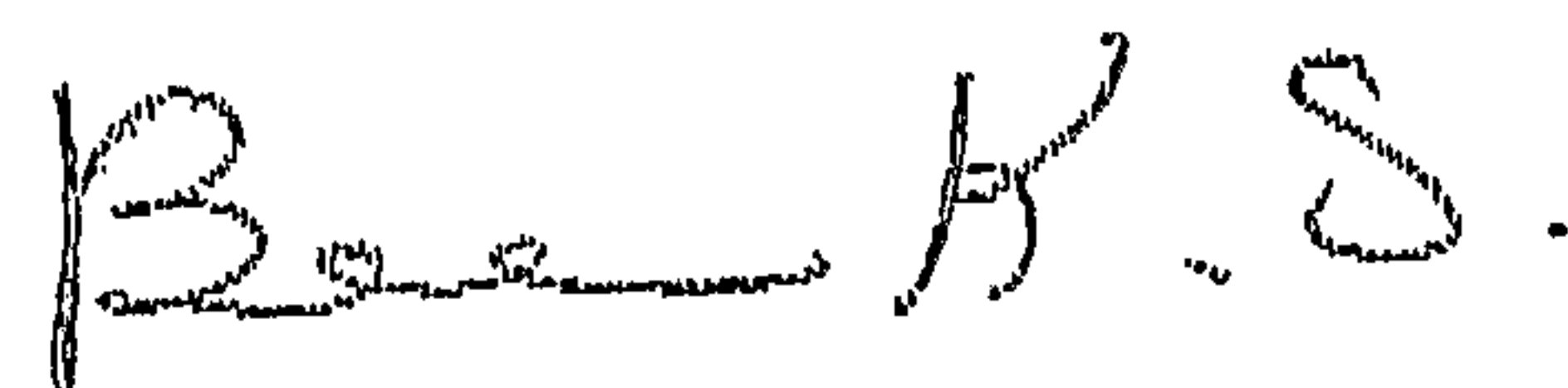
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT
OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : HONG, Hyo Jeong
Clover Apt. 117-201, Dunsan-dong, Seo-ku, Taejon 302-772,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DH5 α /p α CMV-dhfrC-HuKRI27	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCTC 10198BF
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☒ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on March 13 2002 .	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on _____ and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-383, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):  BAE, Kyung Sook, Director Date: March 16 2002

31

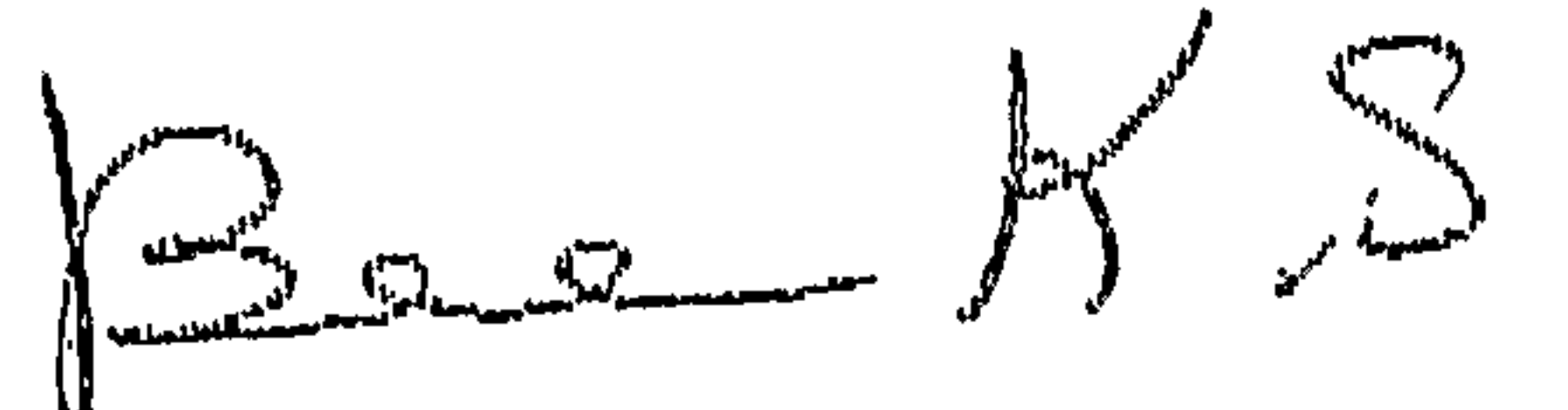
BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT
OF MICROORGANISMS FOR THE PURPOSE OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

issued pursuant to Rule 7.1

TO : HONG, Hyo Jeong
Clover Apt. 117-201, Dunsan-dong, Seo-ku, Taejeon 302-772,
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: CHO/HuKR127 (CHO cell line)	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCTC 10199BF
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☒ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on March 13 2002.	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on _____ and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejeon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of authorized official(s):  BAE, Kyung Sook, Director Date: March 16 2002

SEQUENCE LISTING

<110> APROGEN, INC.

<120> HUMANIZED ANTIBODY AND PROCESS FOR PREPARING SAME

<130> 13266-7

<140> CA 2,492,671

<141> 2003-03-22

<150> KR 10-2002-0015708

<151> 2002-03-22

<160> 38

<170> KopatentIn 1.71

<210> 1

<211> 345

<212> DNA

<213> Artificial Sequence

<220>

<223> HEAVY CHAIN of HZVII

<400> 1

```

caggtccagc tgggtgcagtc tggagctgaa gtgaagaagc ctggggcctc agtgaagggt      60
tcctgcaaag cttctggcta caccttcacc agtgcttgga tgaactgggt gcgacaggcc      120
cctggacagg gtcttgagtg gatgggacgg atttatacta gtggtggaag cactagctac      180
gcacagaagt tccagggcag agtcacaatg actgcagaca aatccacgag cacagtctac      240
atggagctca gcagcctgag atctgaggac acggcggtgt attactgtgc aagagagtac      300
cgggttgccc gttggggcca aggaactctg gtcactgtct cttca                      345

```

<210> 2

<211> 115

<212> PRT

<213> Artificial Sequence

<220>

<223> HEAVY CHAIN of HZVII

<400> 2

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Ala Pro Gly Ala
 1           5           10           15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Ala
           20           25           30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
           35           40           45
Gly Arg Ile Tyr Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
 50           55           60

```

33

Gln Gly Arg Val Thr Met Thr Ala Asp Lys Ser Thr Ser Thr Val Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Tyr Arg Val Ala Arg Trp Gly Gln Gly Thr Leu Val Thr
100 105 110

Val Ser Ala
115

<210> 3
<211> 336
<212> DNA
<213> Artificial Sequence

<220>
<223> LIGHT CHAIN of HZVII

<400> 3
gatatcgtga tgacccaaac tccactttct ttgtcgggta cccctggaca accagcctct 60
atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaattgg 120
ttattacaga agccaggcca gcctccacag cgcctaattct atctgggtgtc taatcgggac 180
tctggagtcc ctgacagggtt cagtggcagt ggatcaggaa cagattttac actgaaaatc 240
agcagagtgg aggctgagga tgttggagtt tattactgcg tgcaagggtac acattttcct 300
cagacgttcg gtggaggcac caaggtggaa atcaaa 336

<210> 4
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> LIGHT CHAIN of HZVII

<400> 4
Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30
Asn Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Lys Pro Gly Gln Pro
35 40 45
Pro Gln Arg Leu Ile Tyr Leu Val Ser Asn Arg Asp Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

34

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
 85 90 95

Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

<210> 5
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ryu94

<400> 5
 gagaattcac attcacgatg tacttg

26

<210> 6
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR43-1

<400> 6
 ctgctgcagc tggacctgac tctggacacc att

33

<210> 7
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR44-1

<400> 7
 caggtccagc tgcagcagtc tggacctgaa ctg

33

<210> 8
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR45-1

<400> 8
 tggggcccttg gtggaggctg cagagacagt gac

33

35

<210> 9
 <211> 33
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR46-1

<400> 9
 gcctccacca agggcccatc ggtcttcccc ctg

33

<210> 10
 <211> 28
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR31

<400> 10
 cagcggccgc tcatttaccc ggggacag

28

<210> 11
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Ryu86

<400> 11
 caaagcttgg aagcaagatg gattca

26

<210> 12
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR48

<400> 12
 caagatatcc ccacaggtac cagatac

27

<210> 13
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HUR49

<400> 13
tgtggggata tcttgatgac ccaaact 27

<210> 14
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> HUR50

<400> 14
cacagatctt ttgatttcca gcttggt 27

<210> 15
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> HUR51

<400> 15
atcaaaagat ctgtggctgc accatct 27

<210> 16
<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> CK1D

<400> 16
gcgccgtcta gaattaacac tctcccctgt tgaagctctt tgtgacgggc gaactcag 58

<210> 17
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> YM001N

<400> 17
ccggaattca cattcacgat gtacttg 27

<210> 18
<211> 16
<212> DNA
<213> Artificial Sequence

<220>

<223> YM003

<400> 18

tgccccccaga ggtgct

16

<210> 19

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> ym257

<400> 19

acgcattcag tgcttcttgg atgaactggg tga

33

<210> 20

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> YM258

<400> 20

atccaagaag cactgaatgc gtagccagaa g

31

<210> 21

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> YM004

<400> 21

ccaattcaaa gcggttttttc cattactata taagagggc

38

<210> 22

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> YM009

<400> 22

gcagccaccg tacgtttgat ttccaccttg gt

32

38

<210> 23
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Ryu 166

<400> 23
ggatttgtct gcagtcattg tggctctgcc ctggaactt

39

<210> 24
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Hur 37

<400> 24
gacaaatcca cgagcacagt ctacatg

27

<210> 25
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Ryu 118

<400> 25
ctgtggaggc tggcctggct tctgtaataa cca

33

<210> 26
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Ryu 119

<400> 26
ggccagcctc cacagctcct aatctatctg

30

<210> 27
<211> 345
<212> DNA
<213> Artificial Sequence

<220>
<223> KR127VH

39

<400> 27
 caggtccagc tgcagcagtc tggacctgaa ctggtgaagc ctggggcctc agtgaagatt 60
 tcctgcaaag cttctggcta cgcattcagt agttcttgga tgaactgggt gaagcagagg 120
 cctggacagg gtcttgagtg gattggacgg atttatcctg gagatggaga tactaactac 180
 aatgggaagt tcaagggcaa ggccacactg actgcagaca aatcctccag cacagcctac 240
 atgcagctca gcagcctgac ctctgtggac tctgcggtct atttctgtgc aagagagtac 300
 gacgaggctt actggggcca agggactctg gtcactgtct ctgca 345

<210> 28
 <211> 115
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> KR127VH

<400> 28
 Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
 20 25 30
 Trp Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Arg Ile Tyr Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe
 50 55 60
 Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Thr Ser Val Asp Ser Ala Val Tyr Phe Cys
 85 90 95
 Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val Ser Ala
 115

<210> 29
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> KR127VK

<400> 29
 gatattcttga tgacccaaac tccacttatt ttgtcgggta ccattggaca accagcctct 60

40

```

atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaattgg      120
ttattacaga ggccaggcca gtctccaaag cgcctaattct atctggtgtc taaactggac      180
tctggagtcc ctgacagggt cactggcagt ggatcaggaa cagattttac actgaaaatc      240
atcagagtgg aggctgagga tttgggagtt tattactgcg tgcaaggtag acattttcct      300
cagacgttcg gtggaggcac caagctggaa atcaaa                                336

```

```

<210>      30
<211>      112
<212>      PRT
<213>      Artificial Sequence

```

```

<220>
<223>      KR127VK

```

```

<400>      30
Asp Ile Leu Met Thr Gln Thr Pro Leu Ile Leu Ser Val Thr Ile Gly
  1              5              10              15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
              20              25              30
Asn Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg Pro Gly Gln Ser
              35              40              45
Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
              50              55              60
Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
              65              70              75              80
Ile Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr Cys Val Gln Gly
              85              90              95
Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
              100             105             110

```

```

<210>      31
<211>      294
<212>      DNA
<213>      Artificial Sequence

```

```

<220>
<223>      DP7

```

```

<400>      31
caggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaagggt      60
tcctgcaagg catctggata caccttcacc agctactata tgcactgggt gcgacaggcc      120
cctggacaag ggcttgagtg gatgggaata atcaacccta gtggtggtag cacaagctac      180

```

41

gcacagaagt tccagggcag agtcaccatg accagggaca cgtccacgag cacagtctac 240
 atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gaga 294

<210> 32
 <211> 98
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DP7

<400> 32
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30
 Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45
 Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 33
 <211> 302
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> DPK12

<400> 33
 gatattgtga tgaccagac tccactctct ctgtccgtca cccctggaca gccggcctcc 60
 atctcctgca agtctagtca gagcctcctg catagtgatg gaaagaccta tttgtattgg 120
 tacctgcaga agccaggcca gcctccacag ctctgatct atgaagtttc caaccggttc 180
 tctggagtgc cagatagggt cagtggcagc gggtcaggga cagatttcac actgaaaatc 240
 agccgggtgg aggctgagga tgttgggggt tattactgca tgcaaagtat acagcttcct 300
 cc 302

<210> 34

42

<211> 100
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> DPK12

<400> 34
 Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
 1 5 10 15
 Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
 20 25 30
 Asp Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Pro
 35 40 45
 Pro Gln Leu Leu Ile Tyr Glu Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80
 Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
 85 90 95
 Ile Gln Leu Pro
 100

<210> 35
 <211> 345
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> HEAVY CHAIN of HZI

<400> 35
 caggtccagc tgggtgcagtc tggagctgaa gtggtgaagc ctggggcctc agtgaagggt 60
 tcctgcaaag cttctggcta cgcattcagt agttcttgga tgaactgggt gcgacaggcc 120
 cctggacagg gtcttgagtg gattggacgg atttatcctg gagatggaga tactaactac 180
 gcacagaagt tccagggcaa ggccacactg actgcagaca aatccacgag cacagcctac 240
 atggagctca gcagcctgag atctgaggac acggcggtct atttctgtgc aagagagtac 300
 gacgaggctt actggggcca aggaactctg gtcactgtct cttca 345

<210> 36
 <211> 115
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> HEAVY CHAIN of HZI

43

<400> 36
 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Ser
 20 25 30
 Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 Gly Arg Ile Tyr Pro Gly Asp Gly Ser Thr Ser Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
 85 90 95
 Ala Arg Glu Tyr Asp Glu Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr
 100 105 110
 Val Ser Ser
 115

<210> 37
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> LIGHT CHAIN of HZI

<400> 37
 gatatcttga tgacccaaac tccactttct ttgtcgggta cccctggaca accagcctct 60
 atctcttgca agtcaagtca gagcctctta tatagtaatg gaaaaaccta tttgaattgg 120
 ttattacaga agccaggcca gtctccaaag cgcctaattct atctgggtgtc taaactggac 180
 tctggagtcc ctgacagggtt cagtggcagt ggatcaggaa cagattttac actgaaaatc 240
 agcagagtgg aggctgagga tgttggagtt tattactgcg tgcaagggtac acattttcct 300
 cagacgttcg gtggaggcac caaggtggaa atcaaa 336

<210> 38
 <211> 112
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> LIGHT CHAIN of HZI

<400> 38

Asp Ile Leu Met Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly
1 5 10 15
Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30
Asn Gly Lys Thr Tyr Leu Tyr Trp Leu Leu Gln Lys Pro Gly Gln Ser
35 40 45
Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp Ser Gly Val Pro
50 55 60
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Val Gln Gly
85 90 95
Thr His Phe Pro Gln Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

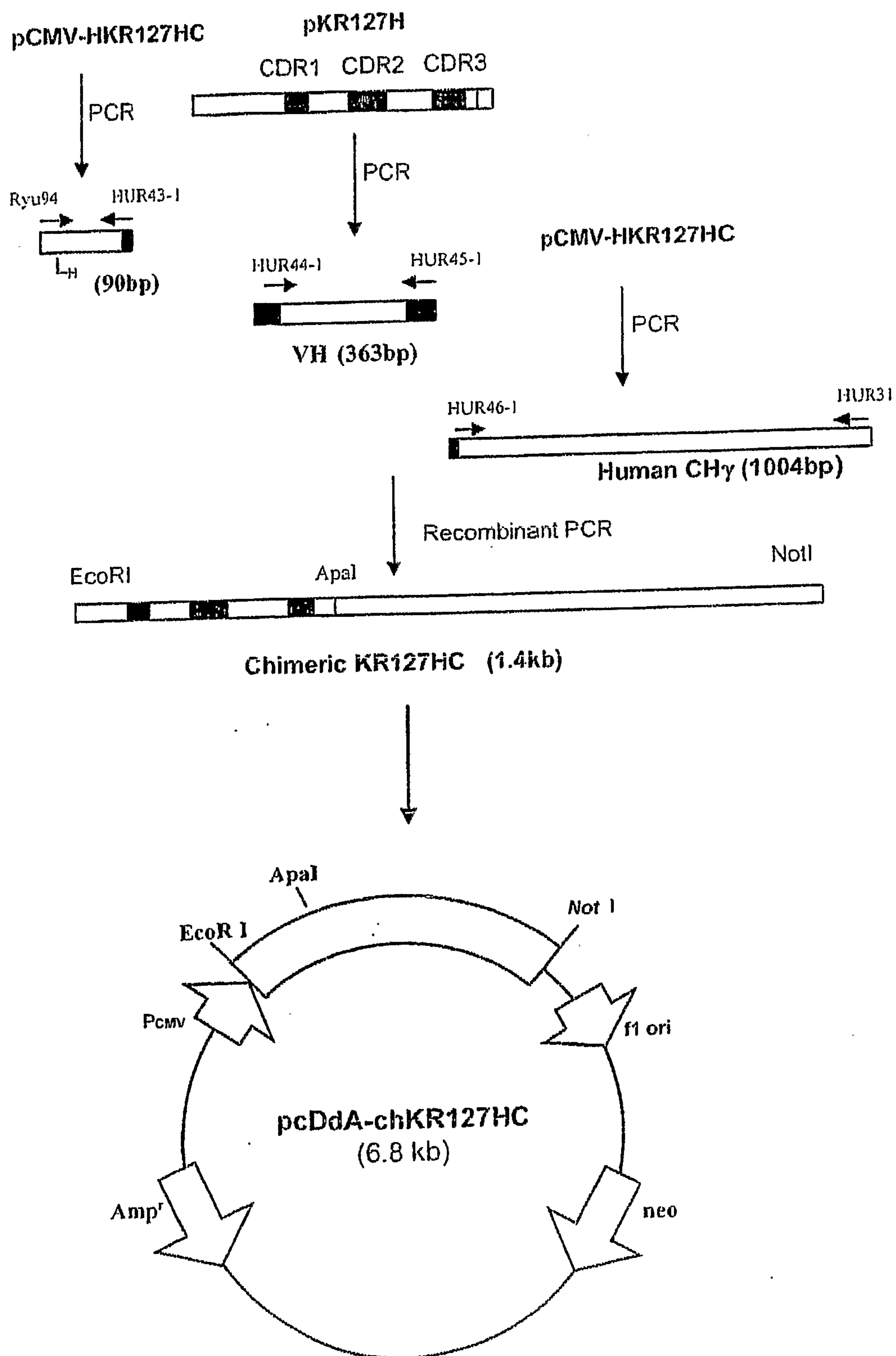
We Claim:

1. A process for preparing a humanized antibody comprising the steps of:
 - (a) selecting a specificity determining residue (SDR) of the complementarity determining region (CDR) of murine monoclonal antibody heavy chain and light chain variable regions, wherein the step (a) is conducted by replacing each of the amino acid residues of CDR with alanine to produce mutants, selecting a mutant that has lower affinity (K_D) to a human antigen than the original murine antibody and determining the replaced amino acid residue of said mutant as a SDR; and
 - (b) grafting all of the said SDR to the corresponding amino acids of human antibody variable regions.
2. A humanized antibody produced by CHO/HuKR127 (Accession No.: KCTC10199BP).
3. A DNA encoding a humanized antibody heavy chain comprising the amino acid sequence of SEQ ID NO:2.
4. The DNA of Claim 3, wherein the variable region comprises the nucleotide sequence of SEQ ID NO:1.
5. A DNA encoding a humanized antibody light chain comprising the amino acid sequence of SEQ ID NO: 4.
6. The DNA of Claim 5, wherein the variable region comprises the nucleotide sequence of SEQ ID NO:3.
7. An expression vector pHuKR127HC comprising the DNA of Claim 4.
8. An expression vector pHuKR127KC comprising the DNA of Claim 6.

9. An expression vector pdCMV-dhfrC-HuKR127 comprising both the DNAs of Claim 4 and Claim 6.
- 5 10. An *E. coli* DH5 α /pdCMV-dhfrC-HuKR127 (Accession No.: KCTC 10198BP) transformed with the expression vector of Claim 9.
11. CHO cell line CHO/HuKR127 (Accession No.: KCTC 10199BP) producing the humanized antibody of Claim 2.
- 10
12. A composition for preventing or treating HBV infection comprising the humanized antibody of Claim 2 and a pharmaceutically acceptable carrier.
13. A use of the humanized antibody of claim 2 for preventing or treating
- 15 HBV infection.

1/10

FIG. 1



2/10

FIG. 2A

		Q	V	Q	L	Q	Q	S	G	P	E	L	V	K	P	
KR127VH		CAG	GTC	CAG	CTG	CAG	CAG	TCT	GGA	CCT	GAA	CTG	GTG	AAG	CCT	42
		Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	
DP7		CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCT	GAG	GTG	AAG	AAG	CCT	
HZ I		CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGA	GCT	GAA	GTG	GTG	AAG	CCT	
HZ VII		CAG	GTC	CAG	CTG	GTG	CAG	TCT	GGA	GCT	GAA	GTG	AAG	AAG	CCT	
HZ I		-	-	-	-	V	-	-	-	A	-	V	-	-	-	
HZ VII		-	-	-	-	V	-	-	-	A	-	V	K	-	-	
		G	A	S	V	K	I	S	C	K	A	S	G	Y	A	
KR127VH		GGG	GCC	TCA	GTG	AAG	ATT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	GCA	84
		G	A	S	V	K	V	S	C	K	A	S	G	Y	T	
DP7		GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAG	GCA	TCT	GGA	TAC	ACC	
HZ I		GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	GCA	
HZ VII		GGG	GCC	TCA	GTG	AAG	GTT	TCC	TGC	AAA	GCT	TCT	GGC	TAC	ACC	
HZ I		-	-	-	-	-	V	-	-	-	-	-	-	-	-	
HZVII		-	-	-	-	-	V	-	-	-	-	-	-	-	T	
		CDR1														
		F	S	<u>S</u>	<u>S</u>	<u>W</u>	<u>M</u>	<u>N</u>	W	V	K	Q	R	P	G	
KR127VH		TTC	AGT	AGT	TCT	TGG	ATG	AAC	TGG	GTG	AAG	CAG	AGG	CCT	GGA	126
		F	T	S	Y	Y	M	H	W	V	R	Q	A	P	G	
DP7		TTC	ACC	AGC	TAC	TAT	ATG	CAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZ I		TTC	AGT	AGT	TCT	TGG	ATG	AAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZ VII		TTC	ACC	AGT	GCT	TGG	ATG	AAC	TGG	GTG	CGA	CAG	GCC	CCT	GGA	
HZ I		-	-	-	-	-	-	-	-	-	R	-	A	-	-	
HZVII		-	T	-	A	-	-	-	-	-	R	-	A	-	-	
		CDR2														
		Q	G	L	E	W	I	G	<u>R</u>	<u>I</u>	<u>Y</u>	<u>P</u>	<u>G</u>	<u>D</u>	<u>G</u>	
KR127VH		CAG	GGT	CTT	GAG	TGG	ATT	GGA	CGG	ATT	TAT	CCT	GGA	GAT	GGA	168
		Q	G	L	E	W	M	G	I	I	N	P	S	G	G	
DP7		CAA	GGG	CTT	GAG	TGG	ATG	GGA	ATA	ATC	AAC	CCT	AGT	GGT	GGT	
HZ I		CAG	GGT	CTT	GAG	TGG	ATT	GGA	CGG	ATT	TAT	CCT	GGA	GAT	GGA	
HZVII		CAG	GGT	CTT	GAG	TGG	ATG	GGA	CGG	ATT	TAT	CCT	AGT	GGT	GGA	
HZ I		-	-	-	-	-	-	-	-	-	-	-	-	-	-	
HZVII		-	-	-	-	-	M	-	-	-	-	-	S	G	-	

3/10

FIG. 2B

CDR2														
	D	T	N	Y	N	G	K	F	K	G	K	A	T	L
KR127VH	GAT	ACT	AAC	TAC	AAT	GGG	AAG	TTC	AAG	GGC	AAG	GCC	ACA	CTG 210
	S	T	S	Y	A	Q	K	F	Q	G	R	V	T	M
DP7	AGC	ACA	AGC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACC	ATG
HZ1	GAT	ACT	AAC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AAG	GCC	ACA	CTG
HZV11	AGC	ACT	AGC	TAC	GCA	CAG	AAG	TTC	CAG	GGC	AGA	GTC	ACA	ATG
HZ1	-	-	-	-	A	Q	-	-	Q	-	-	-	-	-
HZV11	S	-	S	-	A	Q	-	-	Q	-	R	V	-	M

	T	A	D	K	S	S	S	T	A	Y	M	Q	L	S
KR127VH	ACT	GCA	GAC	AAA	TCC	TCC	AGC	ACA	GCC	TAC	ATG	CAG	CTC	AGC 252
	T	R	D	T	S	T	S	T	V	Y	M	E	L	S
DP7	ACC	AGG	GAC	ACG	TCC	ACG	AGC	ACA	GTC	TAC	ATG	GAG	CTG	AGC
HZ1	ACT	GCA	GAC	AAA	TCC	ACG	AGC	ACA	GCC	TAC	ATG	GAG	CTC	AGC
HZV11	ACT	GCA	GAC	AAA	TCC	ACG	AGC	ACA	GTC	TAC	ATG	GAG	CTC	AGC
HZ1	-	-	-	-	-	T	-	-	-	-	-	E	-	-
HZV11	-	-	-	-	-	T	-	-	V	-	-	E	-	-

	S	L	T	S	V	D	S	A	V	Y	F	C	A	R
KR127VH	AGC	CTG	ACC	TCT	GTG	GAC	TCT	GCG	GTC	TAT	TTC	TGT	GCA	AGA 294
	S	L	R	S	E	D	T	A	V	Y	Y	C	A	R
DP7	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCC	GTG	TAT	TAC	TGT	GCG	AGA
HZ1	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCG	GTC	TAT	TTC	TGT	GCA	AGA
HZV11	AGC	CTG	AGA	TCT	GAG	GAC	ACG	GCG	GTG	TAT	TAC	TGT	GCA	AGA
HZ1	-	-	R	-	E	-	T	-	-	-	-	-	-	-
HZV11	-	-	R	-	E	-	T	-	-	-	Y	-	-	-

CDR3														
	E	Y	D	E	A	Y	W	G	Q	G	T	L	V	T
KR127VH	GAG	TAC	GAC	GAG	GCT	TAC	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACT 336
							W	G	Q	G	T	L	V	T
HZ1	GAG	TAC	GAC	GAG	GCT	TAC	TGG	GGC	CAA	GGA	ACT	CTG	GTC	ACT
HZV11	GAG	TAC	CGG	GTT	GCC	CGT	TGG	GGC	CAA	GGA	ACT	CTG	GTC	ACT
HZ1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HZV11	-	-	R	V	-	R	-	-	-	-	-	-	-	-

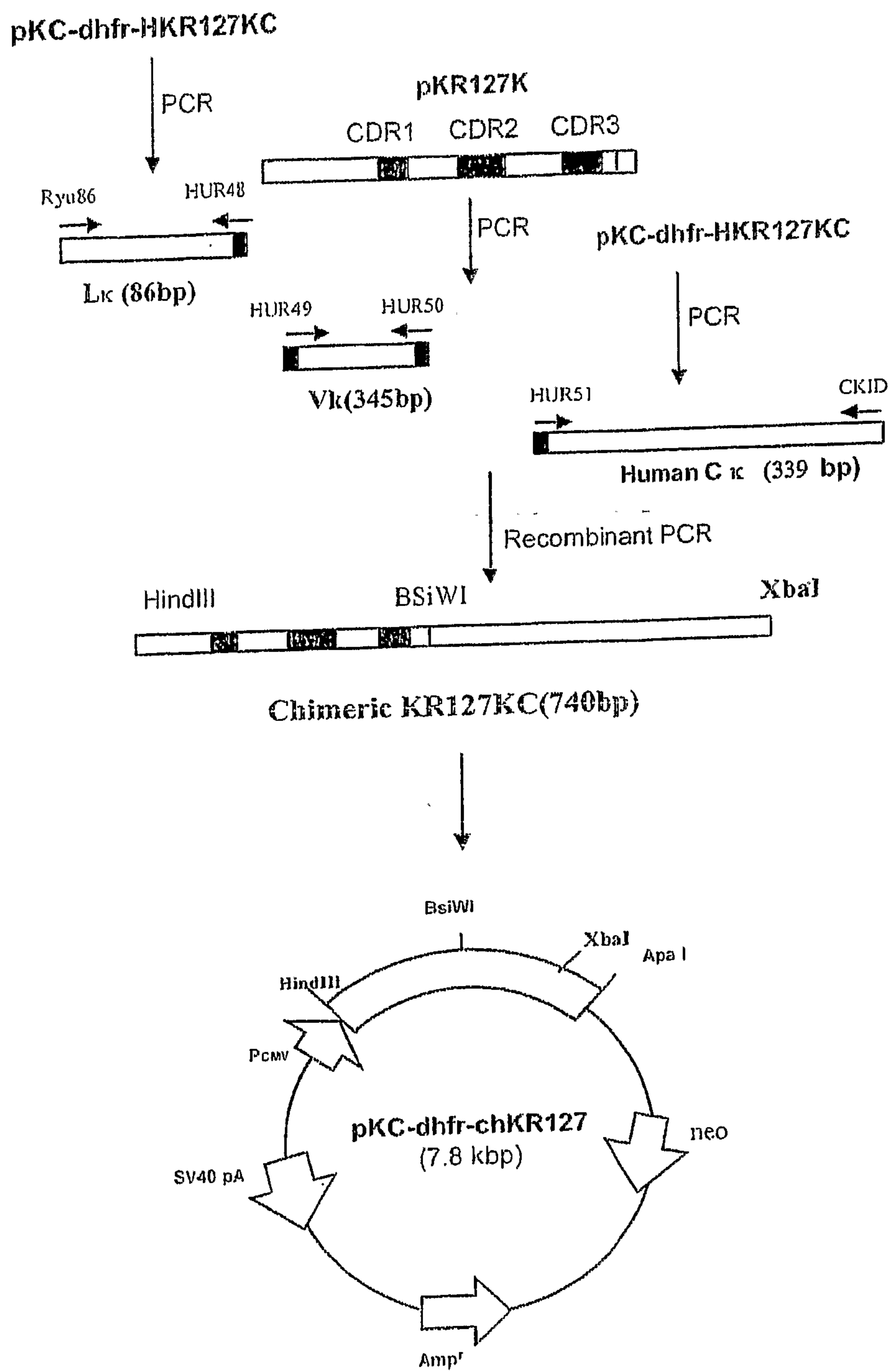
4/10

FIG. 2C

	V	S	A
KR127VH	GTC	TCT	GCA 345
	V	S	S
HZI	GTC	TCT	TCA
HZVII	GTC	TCT	TCA
HZI	-	-	S
HZVII	-	-	S

5/10

FIG. 3



6/10

FIG. 4A

	D	I	L	M	T	Q	T	P	L	I	L	S	V	T	
KR127VK	GAT	ATC	TTG	ATG	ACC	CAA	ACT	CCA	CTT	ATT	TTG	TCG	GTT	ACC	42
	D	I	V	M	T	Q	T	P	L	S	L	S	V	T	
DPK12	GAT	ATT	GTG	ATG	ACC	CAG	ACT	CCA	CTC	TCT	CTG	TCC	GTC	ACC	
HZ1	GAT	ATC	TTG	ATG	ACC	CAA	ACT	CCA	CTT	TCT	TTG	TCG	GTT	ACC	
HZ1V	GAT	ATC	GTG	ATG	ACC	CAA	ACT	CCA	CTT	TCT	TTG	TCG	GTT	ACC	
HZ1	-	-	-	-	-	-	-	-	-	S	-	-	-	-	
HZ1V	-	-	V	-	-	-	-	-	-	S	-	-	-	-	
CDR1															
	I	G	Q	P	A	S	I	S	C	<u>K</u>	<u>S</u>	<u>S</u>	<u>Q</u>	<u>S</u>	
KR127VK	ATT	GGA	CAA	CCA	GCC	TCT	ATC	TCT	TGC	AAG	TCA	AGT	CAG	AGC	84
	P	G	Q	P	A	S	I	S	C	K	S	S	Q	S	
DPK12	CCT	GGA	CAG	CCG	GCC	TCC	ATC	TCC	TGC	AAG	TCT	AGT	CAG	AGC	
HZ1	CCT	GGA	CAA	CCA	GCC	TCT	ATC	TCT	TGC	AAG	TCA	AGT	CAG	AGC	
HZ1V	CCT	GGA	CAA	CCA	GCC	TCT	ATC	TCT	TGC	AAG	TCA	AGT	CAG	AGC	
HZ1	P	-	-	-	-	-	-	-	-	-	-	-	-	-	
HZ1V	P	-	-	-	-	-	-	-	-	-	-	-	-	-	
CDR1															
	<u>L</u>	<u>L</u>	<u>Y</u>	<u>S</u>	<u>N</u>	<u>G</u>	<u>K</u>	<u>T</u>	<u>Y</u>	<u>L</u>	<u>N</u>	<u>W</u>	<u>L</u>	<u>L</u>	
KR127VK	CTC	TTA	TAT	AGT	AAT	GGA	AAA	ACC	TAT	TTG	AAT	TGG	TTA	TTA	126
	L	L	H	S	D	G	K	T	Y	L	Y	W	Y	L	
DPK12	CTC	CTG	CAT	AGT	GAT	GGA	AAG	ACC	TAT	TTG	TAT	TGG	TAC	CTG	
HZ1	CTC	TTA	TAT	AGT	AAT	GGA	AAA	ACC	TAT	TTG	AAT	TGG	TTA	TTA	
HZ1V	CTC	TTA	TAT	AGT	AAT	GGA	AAA	ACC	TAT	TTG	AAT	TGG	TTA	TTA	
HZ1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
HZ1V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
CDR2															
	Q	R	P	G	Q	S	P	K	R	L	I	Y	<u>L</u>	<u>V</u>	
KR127VK	CAG	AGG	CCA	GGC	CAG	TCT	CCA	AAG	CGC	CTA	ATC	TAT	CTG	GTG	168
	Q	K	P	G	Q	P	P	Q	L	L	I	Y	E	V	
DPK12	CAG	AAG	CCA	GGC	CAG	CCT	CCA	CAG	CTC	CTG	ATC	TAT	GAA	GTT	
HZ1	CAG	AAG	CCA	GGC	CAG	TCT	CCA	AAG	CGC	CTA	ATC	TAT	CTG	GTG	
HZ1V	CAG	AAG	CCA	GGC	CAG	CCT	CCA	CAG	CGC	CTA	ATC	TAT	CTG	GTG	
HZ1	-	K	-	-	-	-	-	-	-	-	-	-	-	-	
HZ1V	-	K	-	-	-	P	-	Q	-	-	-	-	-	-	

7/10

FIG. 4B

CDR2

	<u>S</u>	<u>K</u>	<u>L</u>	<u>D</u>	<u>S</u>	G	V	P	D	R	F	T	G	S
KR127VK	TCT	AAA	CTG	GAC	TCT	GGA	GTC	CCT	GAC	AGG	TTC	ACT	GGC	AGT 210
	S	N	R	F	S	G	V	P	D	R	F	S	G	S
DPK12	TCC	AAC	CGG	TTC	TCT	GGA	GTG	CCA	GAT	AGG	TTC	AGT	GGC	AGC
HZ1	TCT	AAA	CTG	GAC	TCT	GGA	GTC	CCT	GAC	AGG	TTC	AGT	GGC	AGT
HZ1V	TCT	AAT	CGG	GAC	TCT	GGA	GTC	CCT	GAC	AGG	TTC	AGT	GGC	AGT
HZ1	-	-	-	-	-	-	-	-	-	-	-	S	-	-
HZ1V	-	N	R	-	-	-	-	-	-	-	-	S	-	-

	G	S	G	T	D	F	T	L	K	I	I	R	V	E
KR127VK	GGA	TCA	GGA	ACA	GAT	TTT	ACA	CTG	AAA	ATC	ATC	AGA	GTG	GAG 252
	G	S	G	T	D	F	T	L	K	I	S	R	V	E
DPK12	GGG	TCA	GGG	ACA	GAT	TTC	ACA	CTG	AAA	ATC	AGC	CGG	GTG	GAG
HZ1	GGA	TCA	GGA	ACA	GAT	TTT	ACA	CTG	AAA	ATC	AGC	AGA	GTG	GAG
HZ1V	GGA	TCA	GGA	ACA	GAT	TTT	ACA	CTG	AAA	ATC	AGC	AGA	GTG	GAG
HZ1	-	-	-	-	-	-	-	-	-	-	S	-	-	-
HZ1V	-	-	-	-	-	-	-	-	-	-	S	-	-	-

CDR3

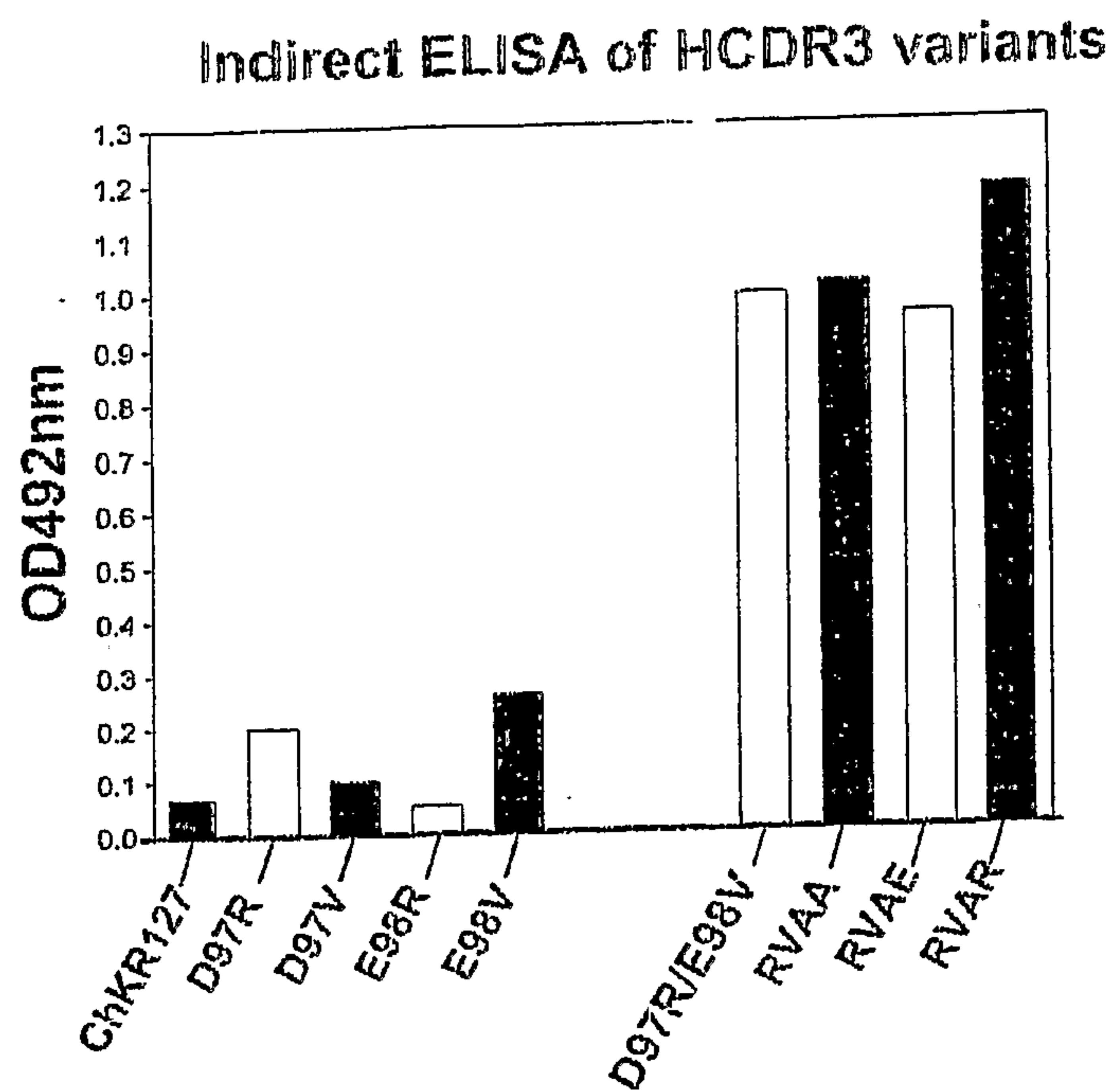
	A	E	D	L	G	V	Y	Y	C	<u>V</u>	<u>Q</u>	<u>G</u>	<u>T</u>	<u>H</u>
KR127VK	GCT	GAG	GAT	TTG	GGA	GTT	TAT	TAC	TGC	GTG	CAA	GGT	ACA	CAT 294
	A	E	D	V	G	V	Y	Y	C	M	Q	S	I	Q
DPK12	GCT	GAG	GAT	GTT	GGG	GTT	TAT	TAC	TGC	ATG	CAA	AGT	ATA	CAG
HZ1	GCT	GAG	GAT	GTT	GGA	GTT	TAT	TAC	TGC	GTG	CAA	GGT	ACA	CAT
HZ1V	GCT	GAG	GAT	GTT	GGA	GTT	TAT	TAC	TGC	GTG	CAA	GGT	ACA	CAT
HZ1	-	-	-	V	-	-	-	-	-	-	-	-	-	-
HZ1V	-	-	-	V	-	-	-	-	-	-	-	-	-	-

CDR3

	<u>F</u>	<u>P</u>	<u>Q</u>	<u>T</u>	F	G	G	G	T	K	L	E	I	K
KR127VK	TTT	CCT	CAG	ACG	TTC	GGT	GGA	GGC	ACC	AAG	CTG	GAA	ATC	AAA 336
	L	P		T	F	G	G	G	T	K	V	E	I	K
DPK12	CTT	CCT	CC											
														(JK4)
HZ1	TTT	CCT	CAG	ACG	TTC	GGT	GGA	GGC	ACC	AAG	GTG	GAA	ATC	AAA
HZ1V	TTT	CCT	CAG	ACG	TTC	GGT	GGA	GGC	ACC	AAG	GTG	GAA	ATC	AAA
HZ1	-	-	-	-	-	-	-	-	-	-	V	-	-	-
HZ1V	-	-	-	-	-	-	-	-	-	-	V	-	-	-

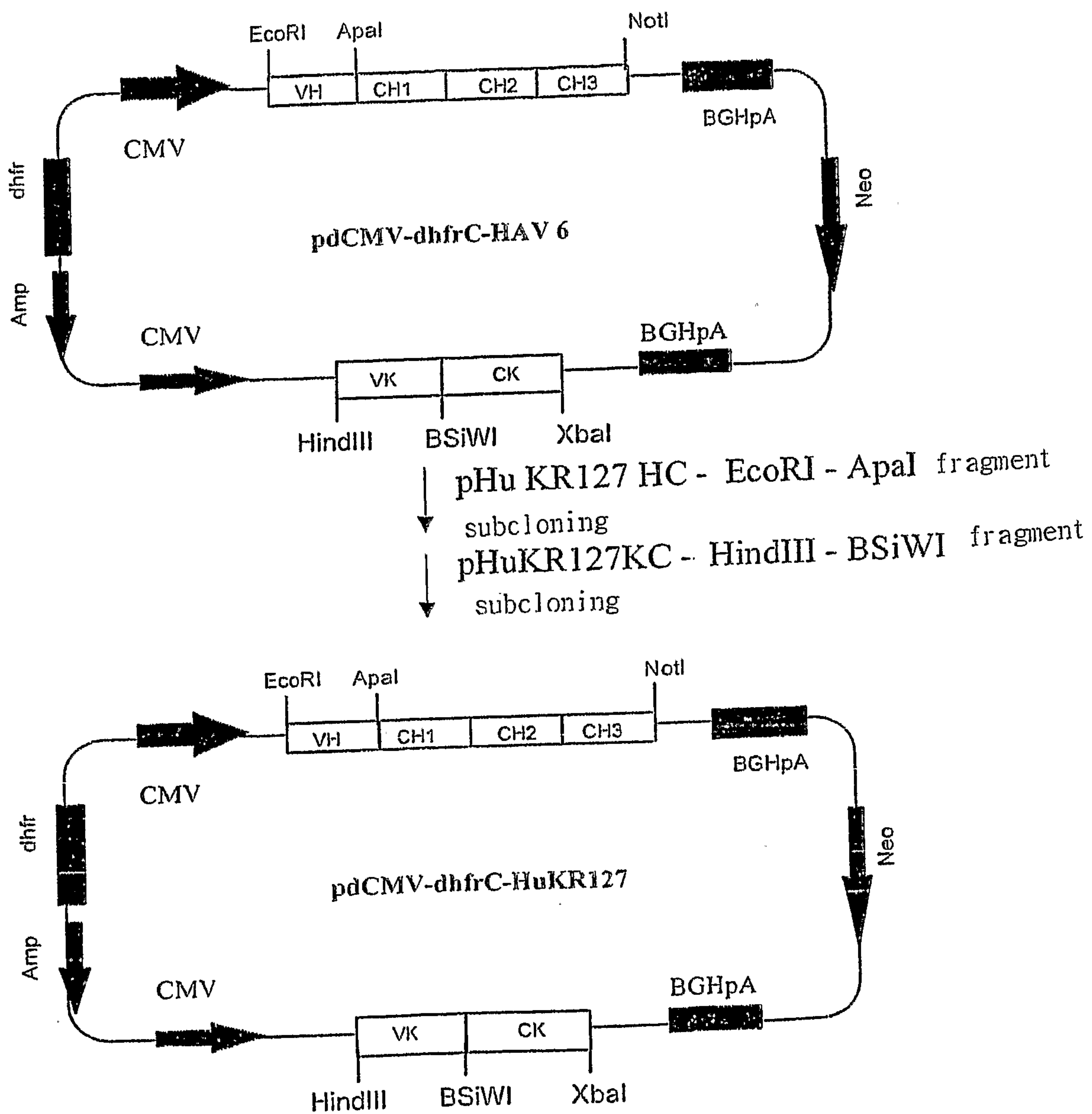
8/10

FIG. 5

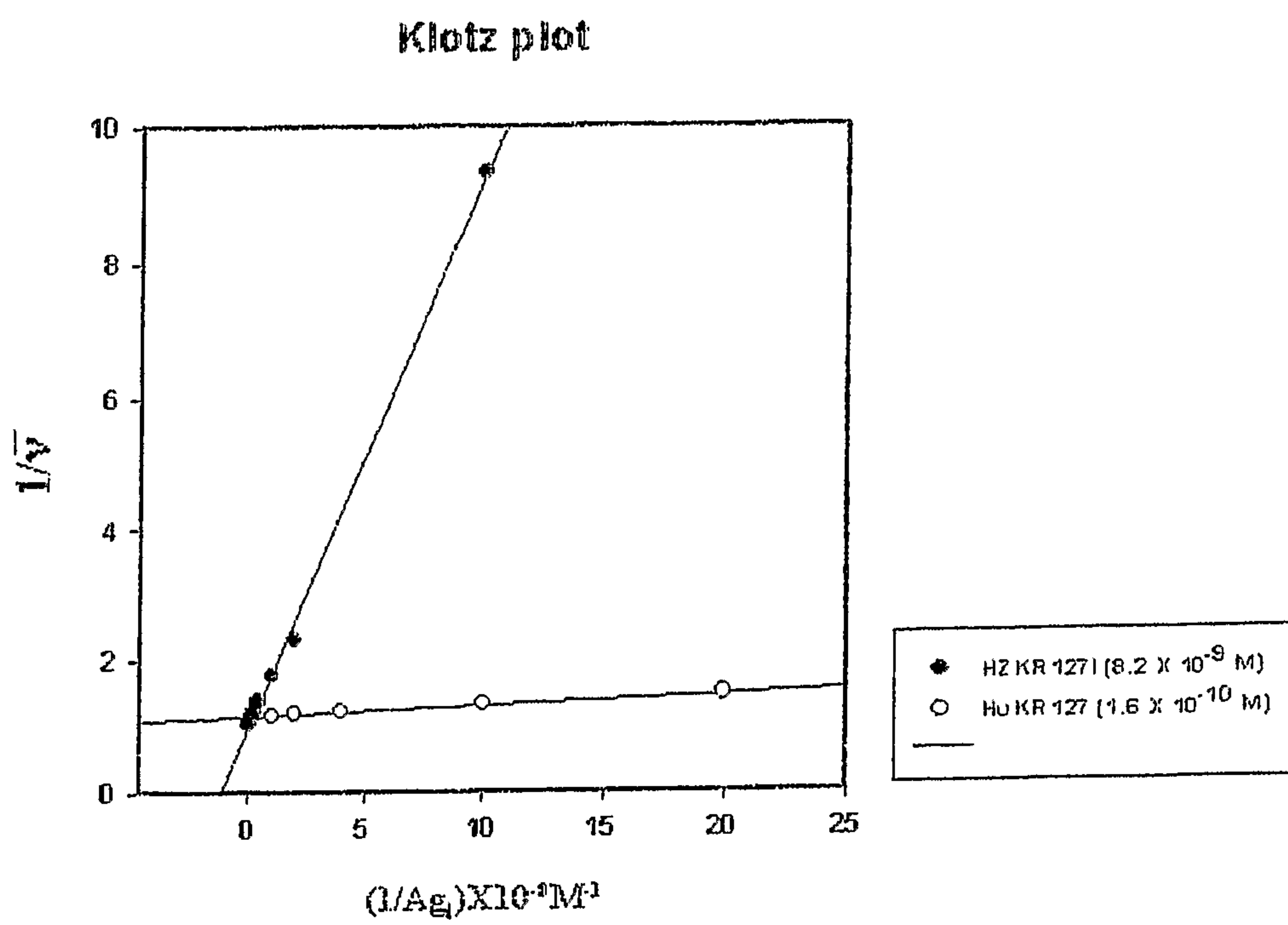


9/10

FIG. 6



10/10
FIG. 7



Klotz plot

