

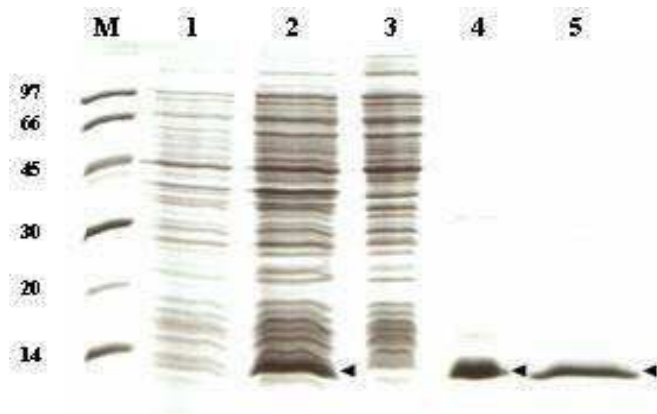
	(19) (12)	(KR) (A)	(11) (43)	10-2010-0041105 2010 04 22			
(51)	Int. Cl.		(71)				
	C07K 19/00 (2006.01) C07K 14/485 (2006.01)			100			
	C07K 14/78 (2006.01)		(72)				
(21)	10-2008-0100116						
(22)	2008 10 13			1		10	
	2008 10 13		1 204				
				3		101	1505
			(	)			
			(74)				
	:		8				

(54)

(57)

Vibrio mimicus

- 1



(72)

				2	1	4
		1200		1303		
110	1101					

1

1 , .

2

1 , (*Vibrio mimicus*)

.

3

(*Vibrio mimicus*)

1 .

4

3 , 2 .

5

3 .

6

5 .

7

6 .

8

1 , .

[0001] *Vibrio mimicus* ,

.

[0002]

(hEGF) 6kDa 53

[0003]

(MMPs)

. MMP

C

C-

C-

(CBD)

[0004]

Vibrio alginolyticus(VAC), *Vibrio anguillarum*(EmpA), *Vibrio cholerae*(Hap VCC), *Vibrio fluvialis*(VFP), *Vibrio parahaemolyticus*(VPPRT VppC), *Vibrio proteolyticus*(Nprv) *Vibrio vulnificus*(VVP) . *Vibrio mimicus* 31kDa VMP

61kDa VMC (Lee, JH *et al.*, *Biochim. Biophys. Acta* 1384:1, 1998, Chowdhury, MAR *et al.*, *Infect. Immun.*, 58:4159, 1990). VMC (VMC61)

Vibrio cholerae VCC , *Vibrio alginolyticus* VAC

Vibrio parahaemolyticus VPPRT (Lee, J.H. *et al.*, *FEMS Microbiol. Lett.*, 223:293, 2003), *Vibrio mimicus* VMP *Vibrio cholerae* Hap .

[0005]

VMC61 C 100

67

VMC61

(Lee, J.H. *et al.*, *FEMS Microbiol. Lett.*, 223:293, 2003), VMC61 C 33

VMC61 CBD(Collagen binding domain)

(Lee, JH *et al.*,

FEBS Lett., 579:2507, 2005).

[0006]

EGF

, fibronectin-collagen-binding

EGF

[0007]

Chlostridium histolyticum

CBD(collagen binding domain) EGF

EGF

(Nishi N. *et al.*,

Proc. Natl. Acad. Sci. USA, 95:7018, 1998), *C. histolyticum*

200

53

[0008]

, EGF

, EGF

V. mimicus metalloprotease

(3).

[0024]

(5).

[0025]

(*Vibrio mimicus*)

1

[0026]

2

[0027]

E. coli DH5 α , *E. coli* JM101, *E. coli* K12, *E. coli* W3110, *E. coli* X1776, *E. coli* XL-1Blue(Stratagene), *E. coli* B, *E. coli* BL21 .

[0028]

[0029]

[0030]

[0031]

[0032]

(calcium carbonate), (sucrose) (lactose),

(propylene glycol),

[0033]

[0034]

, " (vector)" DNA
 DNA DNA .

" (plasmid)" " (vector)"

(a)

, (b)

DNA

(c)

(oligonucleotide adaptor)

(linker)

DNA

(ligation)

[0035]

. *E. coli* DH5 α , *E. coli* JM101, *E. coli* K12, *E. coli* W3110, *E. coli* X1776, *E. coli* XL-1Blue(Stratagene), *E. coli* BL21 . FMB101, NM522, NM538
 NM539 *E. coli* (speices) (genera) . *E. coli* , A4 , (*Bacillus subtilis*)
 (bacilli), (*Salmonella typhimurium*) (*Serratia marcescens*) (*Pseudomonas*)

[0036] Sambrook et al., supra 1.82
 . , (electroporation)(Neumann et al., EMBO J., 1:841, 1982)

[0037] , pET (Novagen) . pET
 ,
 , Ni-NTA His- (Novagen)

[0038] " (expression control sequence)"
 DNA . mRNA
 ,
 ,
 .
 SR α (cytomegalovirus)
 DNA ,
 , SV40 , lac
 , trp , TAC TRC , T3 T7 ,
 , fd , 3- ,
 , Pho5, -

T7 .
 [0039] " (operably linked)" .
 (,) ()
 () . , (pre-sequence) (leader)
 DNA DNA
 ;
 ;
 ;
 " DNA ,
 . (enhancer) .
 ()
 (oligonucleotide adaptor) (linker) .

[0040] " " DNA (recombinant
 carrier) DNA DNA , DNA
 DNA DNA . DNA
 () DNA .

[0041] , ,
 .
 (replication origin)

[0042]

" DNA " DNA "

[0043]

DNA

DNA
 DNA
 DNA
 DNA
 cDNA
 (panning),
 (film emulsion)
 (binding),

[0044]

[0045]

1: hEGF-CBD

[0046]

hEGF-CBD	, hEGF	CBD	PCR
PCR	, hEGF-CBD	.	

[0047]

[illegible]

[0048]

3:5' -GGCCGATCCAATAGTGACTCTGAATGTCCC-3

[0049]

4:5' -AGACAGTACCAAGCGCAGTTCCCACCAC-3'

[0050]

CBD	pETMETA61	PCR	
+	+	+	+
+	+	-	+
+	-	+	+
+	-	-	+
-	+	+	+
-	+	-	+
-	-	+	+
-	-	-	+

[0051]

pETMETA61		,	, PCR		<i>Vibrio mimicus</i>
(vmc)	(AF004832)	pVMC193	,	5	6
PCR	.				

[0052]

PCR	94	10		94	30	, 55	30	72	1.5
	15		72	10	.	PCR		<i>NdeI</i>	<i>BamHI</i>
			pET22b(+)	(Novagen, USA)		, pETMETA61			

[0053] 5: 5'-GGCCCATATGGCAGAACAAGCCCAACGC-3' (F1)

[0054] 6: 5'-GGCCGGATCCCCTGTAAAGATCGGCGTGG-3' (R1)

[0055] pETMETA61, *EcoRI* 7 hEGF
3' 8 PCR .

[0056] 7: 5'-GGCCGAATTCCTATGTATCAAGCCAGACTGCAA-3

[0057] 8: 5'-TGGGAACTGCGGTTGGTACTGTCTCGACCA-3

[0058] EGF CBD 3 7 PCR, 258bp hEGF-CBD
*Bam*HI *EcoRI*, pET22b(+) (Novagen, USA) pET-hEGF-CBD
.

[0059] 2: hEGF-CBD

[0060] 1 pET-hEGF-CBD *E. coli* BL21(DE3), 10 /mL
LB 37 , OD 0.4 , IPTG 1mM , hEGF-CBD
, 37 4 .

[0061] hEGF-CBD *E. coli* BL21(DE3) ,
.

[0062] , 50mM Tris-HCl (pH 8.0)
, 4 , 12,000rpm 10 ,
, 6M 20mM Tris-HCl (pH 8.0) , 20mM Tris-HCl
(pH 8.0) (refolding) . His-binding (Novagen, USA)
hEGF-CBD , .

[0063] SDS-PAGE 1 . 1 , 9 kDa
hEGF-CBD *E. coli* BL21(DE3) (1),
hEGF-CBD (5).

[0064] 3: hEGF-CBD

[0065] hEGF-CBD hEGF .

[0066] A-431(ATCC CRL-1555) 37 5% CO₂ 10% FBS MEM
10% FBS DMEM 6 5x10³ cell/well
, , PBS(phosphate buffered saline) ,
DMEM , 0~100ng/well hEGF(Sigma, USA) hEGF-CBD . 2
, PBS , RPMI(-) , 2 , MTT
.

[0067] , 2 , hEGF-CBD hEGF
, hEGF-CBD hEGF
.

- [0068] **4: EGF**
- [0069] hEGF hEGF-CBD EGF (hEGF
hEGF-CBD) (Sahasrabudde A *et al.*,
Cell stress & Chaperones 11:135, 2006).
- [0070] , A-431 5×10^5 PBS , 100ng hEGF hEGF-CBD 5
, , 10 8% SDS-
PAGE , antiphosphotyrosine
(BioSource,) , .
- [0071] , 3 , hEGF-CBD hEGF EGF
.
- [0072] **5:**
- [0073] (1) GST-CBD GST-CBD -
- [0074] CBD GST(glutathione S-transferase)
, GST .
- [0075] GST-CBD 1 pETMETA61
, *Bam*HI 9 *Xho*I 10
, PCR , CBD .
- [0076] 9:5' -CCGCGTGGATCCTTGGTACTGTCTCGACCA-3'
- [0077] 10:5' -CGGCCGCTCGAGTGTATCAAGCCAGACTGC-3'
- [0078] PCR *Bam*HI *Xho*I , DNA pGEX-4T-1 (Amersham, USA)
GST-CBD pVMCBD (GSTCBD-C) .
E. coli BL21(DE3) . GST-CBD -
, 31kDa .
- [0079] GSTCBD .
- [0080] .
- [0081] 10mg I (Sigma, USA) 5mM CaCl₂ 50mM Tris-
HCl(pH8.0) pre-swelling , 10,000xg 10 pre-swelling
0.1mg GSTCBD 6 , 0.22 Durapore
membrane device(Millipore, USA) 12% SDS-gel .
- [0082] , 4 , GSTCBD ,
.
- [0083] (2) hEGF-CBD
- [0084] hEGF-CBD (1) .
- [0085] , 5 , hEGF-CBD SDS-PAGE
, .
- [0086] hEGF-CBD 0.1% SDS 1
, SDS-PAGE , 5 3 hEGF-CBD

1 *E. coli* BL21(DE3) SDS-PAGE

1 crude extract , 2 , 3 , 4

5 hEGF-CBD .

2	hEGF-CBD	A-431	MMT
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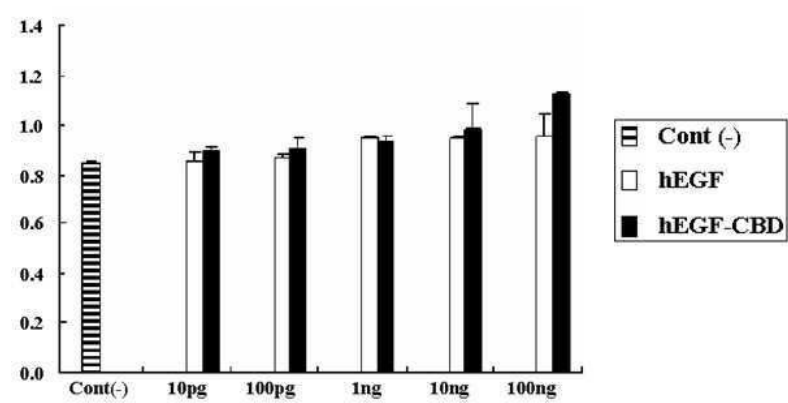
3 (hEGF)

	C	1 hEGF 100ng	2 hEGF-CBD 100ng
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99			
100			

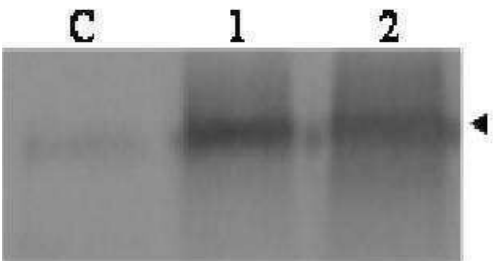
4 GSTCBD , 1 GST-
CBD , 2 GST-CBD .

5, 1
hEGF-CBD, 2 hEGF-CBD, 3, 0.1% SDS
hEGF-CBD.

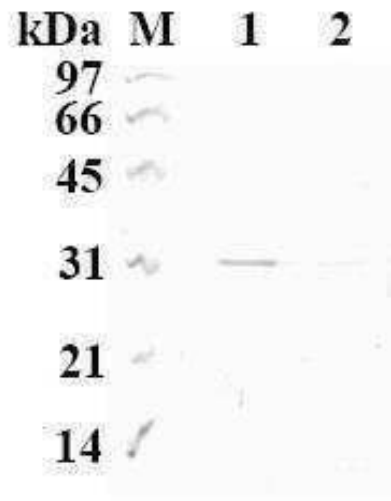
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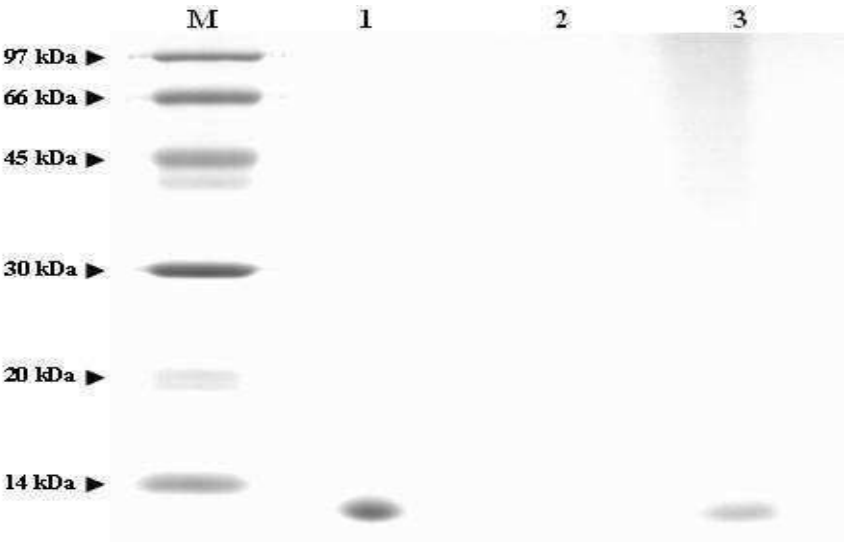
3



4



5



<110> Pukyong National University Industry-University Cooperation Foundation
<120> Fusion Protein Containing Human Epidermal Growth Factor and
Collagen-binding Domain

<130> P08-B158

<160> 10

<170> KopatentIn 1.71

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<211> 85
<212> PRT
<213> Artificial Sequence

<220>
<223> fusion protein hEGF-CBD

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1 5 10 15

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 20 25 30

Val Val Gly Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp
 35 40 45

Trp Glu Leu Arg Leu Val Leu Ser Arg Pro Gly Gln Phe Ala Gln Trp
 50 55 60

Ala Gln Thr Val Lys Asn Leu Gly Glu Gln Tyr Asn Ala Glu Phe Ala
 65 70 75 80

Val Trp Leu Asp Thr
 85

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tgtcagtacc gagacctgaa gtgggtgggaa ctgcgcttgg tactgtctcg accagggcaa 180

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gtctggcttg ataca 255

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28

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<220>
<223> primer

<400> 10

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30