

(19)
(12)

(KR)
(A)

(51) 。 Int. Cl. ⁷
C07K 14/54

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(43)

2002 - 0003917
2002 01 16

(21)
(22)

10 - 2000 - 0035381
2000 06 26

(71)

52

(72)

383 - 2 2 - 305

214 - 6 202

87176 - 1 106 - 1004

(74)

:

(54)

- 16 , ,

- 16

- 16 , - 16 ,

(thioredoxin) , (enterokinas

e) C - C - - 16

- 16 ; 1 HIS - BIND C -

- 16 C - - 16 ; 2 HIS - BIND

- 16 C - - 16 ; .

- 16 , C - - 16

6

1 C - - 16 cDNA .

2 pET32 - b(+) / IL - 16 .

3 .

4 SDS - PAGE .

5 2 HIS - BIND S

DS - PAGE .

6 - 16 .

- 16 , - 16 , - 16

- 16(Interleukin - 16; IL - 16) (concanavalin) A

, CD4 가 T - ,

(Lymphocyte chemoattractant factor; LCF) (Cruikshank & Center, J. Immunology 128, 2569 (1982)).

16 가 , - 16 CD4 가 T - , CD8 -

, - 16 CD4 T - 가

(Cruikshank et al., J. Immunology 155, 2902 (1987)).

(G0) CD4 G1 MHC II IL - 2 - 16

, (atopic asthma), (bullous pemphigoid), (sarcoidosis)

, (airways), (sub mucosa)

, - 16 CD4 T - - 16 가

- 16 CD8 가
 - 16
 CD4
 - 16 cDNA 2,376 bp , 2.6 kb (open reading f
 rame; ORF) , 67 kDa 가 - 16 (Pro - IL - 16)
 (Baier et al., Proc. Natl. Acad. Sci. USA. 94, 5273 (1997)). - 16
 - 3 (caspase - 3) 17 kDa - 16
 8)). 17 kDa - 16 (Zhang et al., J. Biol. Chem. 273, 1144 (199
 가 - 16 CD4
 - 16 , - 16 pQE30
 - B (polymyxin - B)
 (Idziorek et al., Clin. Exp. Immunology, 112, 84 (1998)).
 - 16 , - 16
 (tag) pET15b - 16
 Ni - - 16 (Krautwald, J. Immunology, 1
 60, 5874 (1998)). - 16 N - 20
 - 16
 , - 16 - 16
 - 16
 (thioredoxin) , (ente
 rokinase) C - C - - 16

1 HIS - BIND

2 HIS - BIND

- 16

C -

- 16

C -

- 16

- 16

, C -

(enterokinase)

(tag)

C -

, C -

- 16

가

HIS - BIND

N -

가

- 16

: 1

가

(His.Tag)

6

가

C -

: 2

: 3

. C -

- 16

: 4

가

(Cruikshank et al. PNAS, 91, 5109 (1994))

: 5

, 가

80%

90%

, 가

95%

(" ")

(genetic code)

: 6

C -

- 16

DNA

C -

- 16

- 16

(GenBank)

DNA

M90391

RNA

-

: 7 8
(RT - PCR)

C -

- 16 cDNA

C -

- 16 cDNA

C -

16 cDNA

, C -

- 16 cDNA ,

- 16 cDNA pET - 32b(+) (Novagen) C
 pET32 - b(+) / IL - 16
 AD494(DE3)pLysS(Novagen)
 S - S 3
 pET32 - b(+) / IL - 16 AD494(DE3)/pLysS AD49
 4/pET32b(+) / IL - 16 2000 5 9
 KCTC 0779BP

600 nm 가 0.6 0.8 β - D -
 (isopropyl β - D - thiogalactopyranoside; IPTG) 가
 50% 가 가
 - 16
 HIS - BIND , HIS - BIND (Novagen) 1
 Cl(pH 7.4) 150 mM NaCl , 20 mM Tris -
 - 16 2 HIS - BIND
 - 16

- 16 Jurkat
 - 16
 가
 1:

(Lowry) 200 μ l
 C(A(2% Na₂CO₃, 0.1N NaOH), B1(1% CuSO₄ · 5H₂O) B2(2% NaK tartrate · 4
 H₂O) 100:1:1) 1 Ml 가 10 , 2N - (Folin
 - Ciocalteu's reagent; Sigma) 50 μ l 가 30 (Spectrophotom
 eter: Shimadzu) 550 nm (b
 ovine serum albumin)

2: SDS - PAGE

SDS - PAGE (Laemmli, U. K., Nature, 227, 680 - 685 (1970))
 12% (:N,N' - - - =29.2:0.8), 0.375 M Tris - Cl (pH8.8)
 0.1% (SDS) , (stacking gel) 5% , 0.125 M Tris - C
 l (pH 6.8), 0.1% SDS 5X (Laemmli buffer; 60 mM Tris - Cl; pH 6.8, 25%
 , 2% SDS, 5% β - , 0.1%) 5
 (Coomassie Brilliant Blue) R250(Sigma)

1: C - - 16

(1) C - - 16 cDNA

가 C - - 16 cDNA
 RNA (template) : 7 8 25mer 20mer RT - PCR
 PCR 94 1 50 1 (annealing)
 72 1 (extension) 45 . PCR 1 % 가
 , 가 DNA (Agarose Gel DNA extraction Kit, Quiagen)
 393 bp - 16 cDNA - 16 cDNA HindIII
 , HindIII (thymidine) 가 5' - 3' - pCRIII T
 4 DNA 가 pCRIII/IL - 16 . pCRIII/IL - 16
 DH5 α (Life Technologies, Inc.) , DH5 α /pCRIII/IL - 16

1 C - - 16 cDNA

pCRIII/IL - 16 IL - 16 cDNA (Sanger) (dideoxy sequencin
 g)

(2) C - - 16

C - - 16 , 1 pCR
 III/IL - 16 HindIII - 16 cDNA , HindIII
 pET - 32b(+) (Novagen) T4 가 C - - 16
 : 6 pET32 - b(+) /IL - 16
 pET32 - b(+) /IL - 16 DH5 α (Life Technologies, Inc.)
 - (mini - prep.) (Sambrook et al., Molecular Cloning, 2nd ed., 1989)

2 pET32 - b(+) /IL - 16

pET32 - b(+) /IL - 16 CaCl_2 AD494(DE3)/ pLysS(Novagen)
 AD494/pET32b(+) / IL - 16
 2000 5 9 KCTC 0779BP

2: C - - 16

1 2 AD494/pET32b(+) /IL - 16 50 $\mu\text{g}/\text{Ml}$ (ampicilin) 30 $\mu\text{g}/\text{Ml}$
 (chloramphenicol) LB (1% NaCl, 0.5% 1%) (LAC) 3 Ml
 37 16 . 1 Ml 100 Ml LAC 600 nm
 (optical density; OD)가 0.6 0.8 가 IPTG 1 mM
 가 3 ,

3

3:

(1)

1% β - 1/10 0.02% SDS - PAGE 2 (12 mM Tris - Cl pH 6.8, 5% 7,000 rpm 5 , 0.4% SDS,

(50 mM Tris - Cl pH 8.0, 2 mM EDTA) 가 20 DNA , DNase I 14,000 rpm 15 (Triton) X - 100 1 μ g/M ℓ 가 1/10 TE 가 0.1%가 SDS S - PAGE

(2) SDS - PAGE

1 1 , 2 SDS - PAGE

50 % 100 M ℓ 1 mg

4: - 16

(1)

2 100 M ℓ 7,000 rpm 5 (bind ing buffer; 5 mM , 0.5M NaCl, 20mM Tris - Cl pH 7.9; Novagen) 1 M ℓ , 14,0 00 rpm 30

(2) 1 HIS - BIND

1 ND HIS - BIND , HIS - BIND 2 M ℓ , HIS - BI (Novagen) , 5 M ℓ (packing) , 3 가 , 5 (charge buffer; 50 mM NiSO₄) 가 , 3 가 HIS - BIND , 1 (wash buffer; 60 mM , 0.5 M NaCl 20 mM T , 10 6 (elution buffer; 1 M imidazole, 0.5 M NaCl 20 mM Tris - Cl pH 7.9) 가 6 1 M ℓ 1 가

(3)

2
 , CaCl₂ 2 mM 20 mM Tris - Cl(pH, 7.4) 50 mM NaCl
 , 10 , 0.5 0.001 가 가 (enterokinase, Novagen) 50
 μg methylsulfonyl fluoride) 0.57 mM 가 16 PMSF(Phenyl
 SDS - PAGE 2

4 SDS - PAGE 4 , M
 , 1 , 2 0.001 / 50 μg
 , 3 0.5 / 50 μg
 , 4 10 / 50 μg 4
 , - 16 , - 16
 .

(4) 2 HIS - BIND

3 , - 16 2
 HIS - BIND
 PBS 12
 , 2 SDS - PAGE 1

5 2 HIS - BIND S
 DS - PAGE . 5 M , 1 , 2 2
 HIS - BIND - 16 , 3 2
 HIS - BIND . 5 ,
 - 16 HIS - BIND
 80 % , HIS - BIND

5: - 16

4 4 - 16 RPMI 10 μg/Ml
 20 μl 100 μl 24 - . 1.5x10⁶/Ml jurkat(KCTC H154) 500 μl 8
 μm (Nunc) (tissue insert) 가
 - 16 가 , 5% CO₂, 37 3
 RPMI .

6 - 16 6 , - 1
 6 가 .

, C - - 16
 , - 16 ,
 - 16 가 , HIV .

(57)

1.

(thioredoxin) , (enterokinase)
 (tag) C - C - - 16 .

2.

1 ,
 : 3 .

3.

1 ,
 : 5 .

4.

1 .

5.

4 ,
 : 6 .

6.

4 .

7.

6 ,

pET32 - b(+) / IL - 16.

8.

6 .

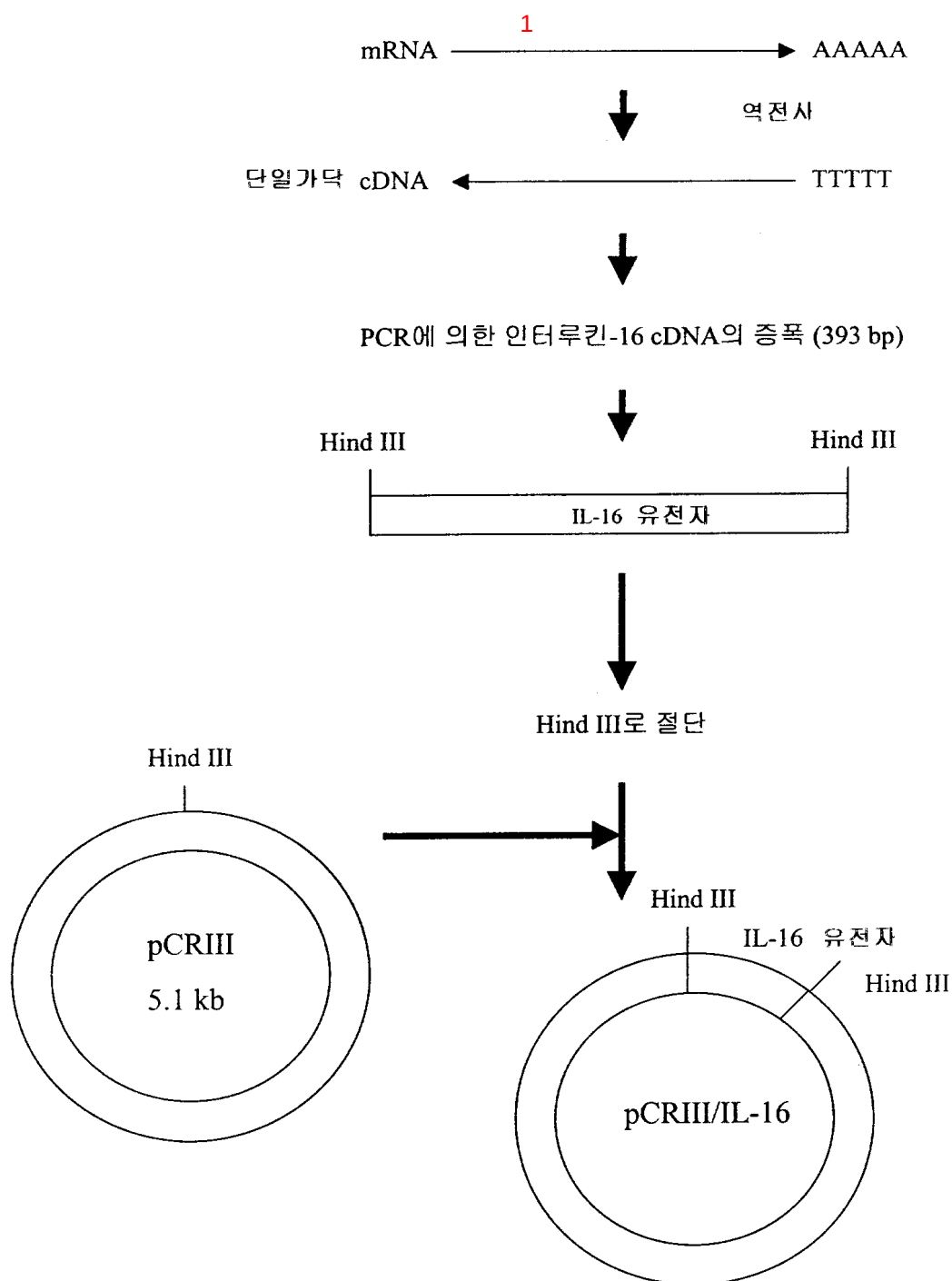
9.

8 ,

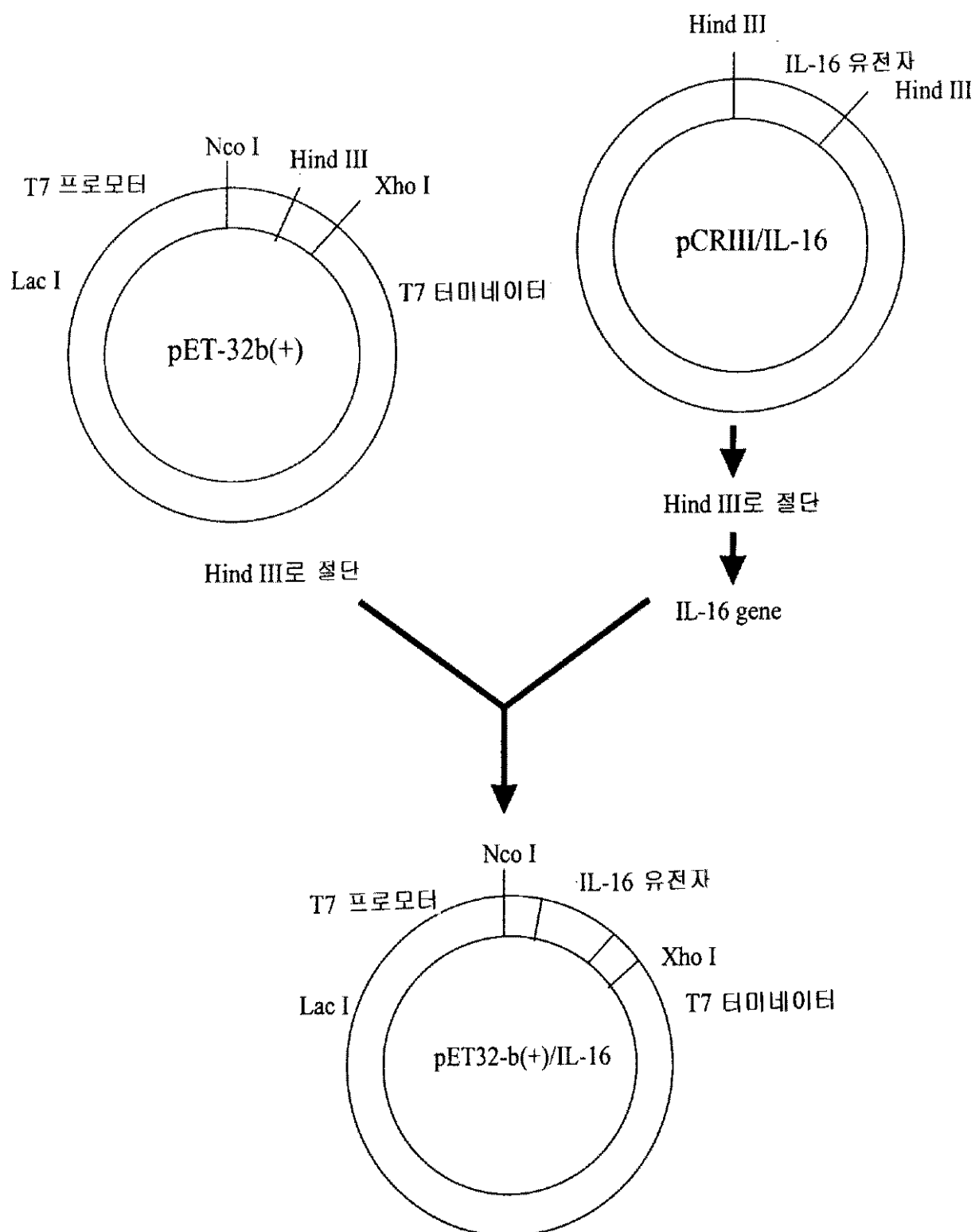
AD494/pET32b(+) / IL - 16(KCTC 0779BP).

10.

8 9 ; 1 HIS - B
IND C - - 16
;
- 16 ; 2 HIS - BIND C - - 16
;
- 16 - 16 ,



2





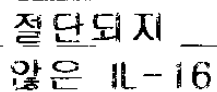
M

1

2

3

4



IL-16

고리
안백절

5

M

1

2

3

[REDACTED]

[REDACTED]

[REDACTED]

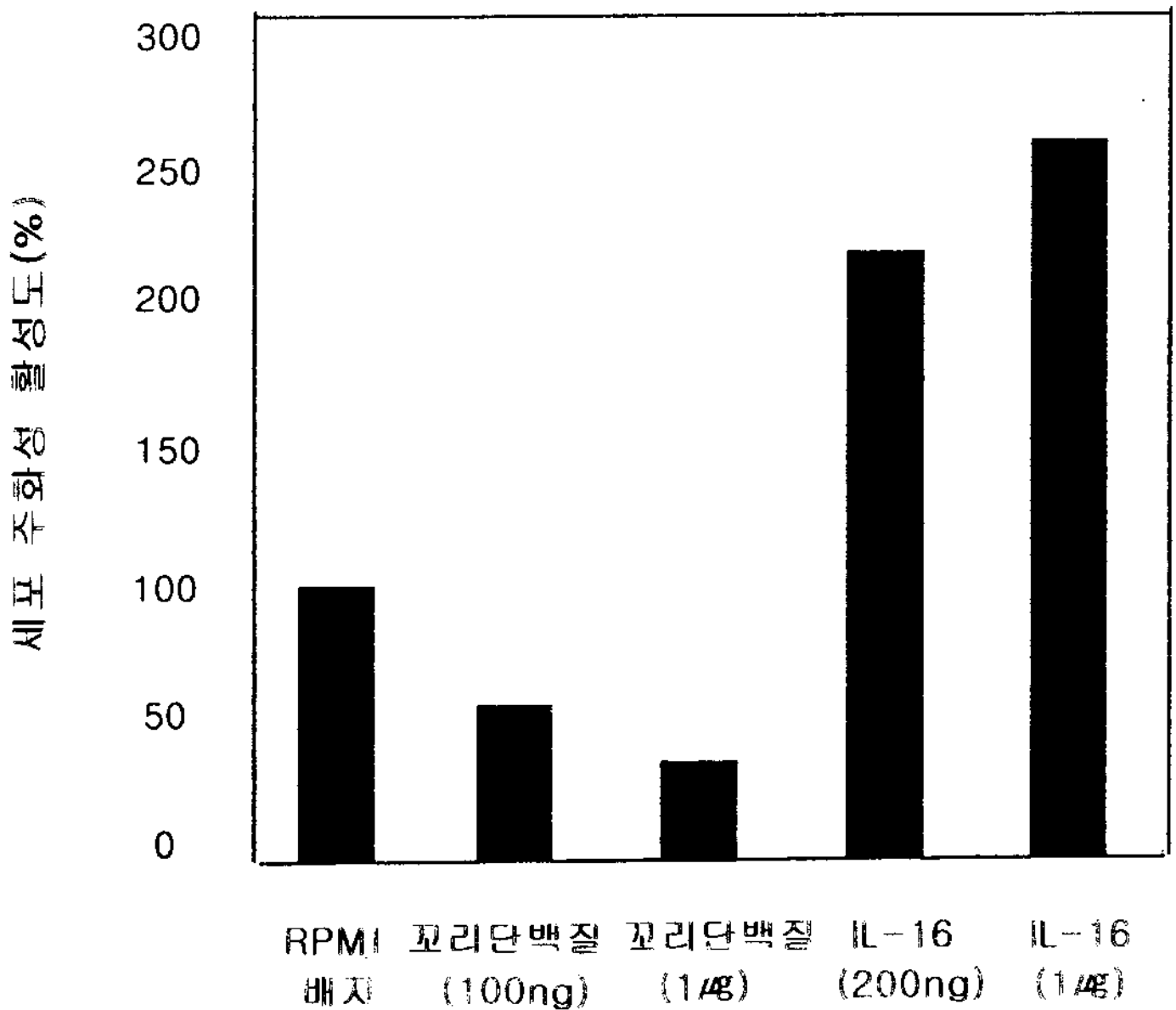
[REDACTED]

rIL-16

꼬리
단백질

[REDACTED]

6



<110> KOREA RESEARCH INSTITUTE OF BIOSCIENCE AND BIOTECHNOLOGY
 <120> FUSION PROTEIN OF TAG PROTEIN AND HUMAN INTERLEUKIN-16, GENE ENCODING SAID FUSION PROTEIN, EXPRESSION VECTOR CONTAINING SAID GENE, E. COLI TRANSFORMED WITH SAID VECTOR AND PROCESS FOR PRODUCING HUMAN INTERLEUKIN-16 USING SAID E. COLI
 <130> 301
 <160> 8
 <170> KOPATIN 1.5
 <210> 1
 <211> 109
 <212> PRT
 <213> Artificial Sequence
 <220>
 <223> THIOREDOXIN

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

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 <211> 5
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 <213> Artificial Sequence
 <220>
 <223> region restricted by enterotoxin
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Asp Asp Asp Asp Lys
 1 5

<210> 3
 <211> 174
 <212> PRT
 <213> Artificial Sequence
 <220>
 <223> tag protein
 <400> 3

Met Ser Asp Lys Ile Ile His Thr Thr Asp Asp Ser Phe Asp Thr Asp
 1 5 10 15
 Val Thr Lys Ala Asp Gly Ala Ile Thr Val Asp Phe Trp Ala Glu Trp
 20 25 30
 Cys Gly Pro Cys Lys Met Ile Ala Pro Ile Thr Asp Glu Ile Ala Asp
 35 40 45
 Glu Trp Gln Gly Lys Thr Thr Val Ala Lys Thr Asn Ile Asp Gln Asn
 50 55 60
 Pro Gly Thr Ala Pro Lys Tyr Gly Ile Arg Gly Ile Pro Thr Thr Thr
 65 70 75 80
 Thr Phe Lys Asn Gly Glu Val Ala Ala Thr Lys Val Gly Ala Thr Ser
 85 90 95
 Lys Gly Gln Leu Lys Glu Phe Thr Asp Ala Asn Thr Ala Gly Ser Gly
 100 105 110
 Ser Gly His Met His His His His His His Ser Ser Gly Leu Val Pro
 115 120 125
 Arg Gly Ser Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln
 130 135 140
 His Met Asp Ser Pro Asp Leu Gly Thr Asp Asp Asp Asp Lys Ala Met

145 150 155 160
 Ala Ile Ser Asp Pro Asn Ser Ser Ser Val Asp Lys Leu Thr
 165 170

<210> 4

<211> 130

<212> PRT

<213> Artificial Sequence

<220>

<223> C-terminal secretory human interleukin-16

<400> 4

Met Pro Asp Leu Asn Ser Ser Thr Asp Ser Ala Ala Ser Ala Ser Ala
 1 5 10 15
 Ala Ser Asp Val Ser Val Glu Ser Thr Ala Glu Ala Thr Val Cys Thr
 20 25 30
 Val Thr Leu Glu Lys Met Ser Ala Gly Leu Gly Phe Ser Leu Glu Gly
 35 40 45
 Gly Lys Gly Ser Leu His Gly Asp Lys Pro Leu Thr Ile Asn Arg Ile
 50 55 60
 Phe Lys Gly Ala Ala Ser Glu Gln Ser Glu Thr Val Gln Pro Gly Asp
 65 70 75 80
 Glu Ile Leu Gln Leu Gly Gly Thr Ala Met Gln Gly Leu Thr Arg Phe
 85 90 95
 Glu Ala Trp Asn Ile Ile Lys Ala Leu Pro Asp Gly Pro Val Thr Ile
 100 105 110
 Val Ile Arg Arg Lys Ser Leu Gln Ser Lys Glu Thr Thr Ala Ala Gly
 115 120 125
 Asp Ser
 130

<210> 5

<211> 304

<212> PRT

<213> Artificial Sequence

<220>

<223> fusion protien of tag protein and C-terminal secretory human inte
 rleukin-16

<400> 5

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

Ser Gly His Met His His His His His His Ser Ser Gly Leu Val Pro
 115 120 125
 Arg Gly Ser Gly Met Lys Glu Thr Ala Ala Ala Lys Phe Glu Arg Gln
 130 135 140
 His Met Asp Ser Pro Asp Leu Gly Thr Asp Asp Asp Lys Ala Met
 145 150 155 160
 Ala Ile Ser Asp Pro Asn Ser Ser Ser Val Asp Lys Leu Thr Met Pro
 165 170 175
 Asp Leu Asn Ser Ser Thr Asp Ser Ala Ala Ser Ala Ser Ala Ala Ser
 180 185 190
 Asp Val Ser Val Glu Ser Thr Ala Glu Ala Thr Val Cys Thr Val Thr
 195 200 205
 Leu Glu Lys Met Ser Ala Gly Leu Gly Phe Ser Leu Glu Gly Gly Lys
 210 215 220
 Gly Ser Leu His Gly Asp Lys Pro Leu Thr Ile Asn Arg Ile Phe Lys
 225 230 235 240
 Gly Ala Ala Ser Glu Gln Ser Glu Thr Val Gln Pro Gly Asp Glu Ile
 245 250 255
 Leu Gln Leu Gly Gly Thr Ala Met Gln Gly Leu Thr Arg Phe Glu Ala
 260 265 270
 Trp Asn Ile Ile Lys Ala Leu Pro Asp Gly Pro Val Thr Ile Val Ile
 275 280 285
 Arg Arg Lys Ser Leu Gln Ser Lys Glu Thr Thr Ala Ala Gly Asp Ser
 290 295 300

<210> 6

<211> 914

<212> DNA

<213> Artificial Sequence

<220>

<223> gene encoding fusion protein of tag protein and C-terminal secretory human interleukin-16

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 gacggggcga tcctcgtcga tttctgggca gagtgggtgc gtccgtgcaa aatgatcgcc 120
 ccgattctgg atgaaatcgc tgacgaatat cagggcaaac tgaccgttgc aaaactgaac 180
 atcgatcaaa accctggcac tgcgccgaaa tatggcatcc gtggtatccc gactctgctg 240
 ctgttcaaaa acgggtgaagt ggcggcaacc aaagtgggtg cactgtctaa aggtcagttg 300
 aaagagttcc tcgacgctaa cctggccggt tctggttctg gccatatgca ccatcatcat 360
 catcattctt ctggtctggt gccacgcggt tctggtatga aagaaaccgc tgctgctaaa 420
 ttcgaacgcc agcacatgga cagcccagat ctgggtaccg acgacgacga caaggccatg 480
 gcgatatcgg atcgaattcg agtccgctc acaagcttac catgcccagac ctcaactcct 540
 ccactgactc tgcagcctca gcctctgcag ccagtgatgt ttctgtagaa tctacagcag 600
 aggccacagt ctgcacggtg aactgggaga agatgtcggc agggctgggc ttcagcctgg 660
 aaggagggaa gggctcccta cacggagaca agcctctcac cattaacagg attttcaaag 720
 gagcagctc agaacaaagt gagacagtcc agcctggaga tgaaatcttg cagctgggtg 780
 gcactgccat gcagggcctc acacggttgg aagcctggaa catcatcaag gcactgcctg 840
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 ctggagactc ctag 914

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<212> DNA
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<400> 7
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<400> 8
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