

(19)
(12)

(KR)
(A)

(51) 。 Int. Cl. ⁷
C07K 14/435

(11)
(43)

2002 - 0008131
2002 01 29

(21)	10 - 2001 - 7011881
(22)	2001 09 18
	2001 09 18
(86)	PCT/EP2000/02117
(86)	2000 03 10

(87)	WO 2000/56885
(87)	2000 09 28

[illegible]

(30)	99105530.2	1999 03 18	EP(EP)
	99109503.5	1999 05 12	EP(EP)

(71)

64293

250

(72)

- 64372	16
- 64283	17
- 64665	42
- 63741	2
- 64404	15
- 64342	5

(74)

:

(54)

12000
cDNA -

가 , 6

udo medicinalis)

가 , 6

12000
cDNA -

(Hir

가 (Ruggeri, Z.M. et al.; Seminars in Hematology, 1994, 31, 229 - 39), 가 (von Willebrand Factor, vWF) vWF, Ib - V - IX ADP 가, 가가 가 IIb/IIIa 가

(Van der Rest M. et al.; FASEB Journal, 1991, 5, 2814 - 23)

가

(version)
(Hirudin), (Hirulog) (Warfarin)
가 .
(Rao, A.K et al., Circulation, 1996, 94, 389 - 2395).

가

ADP (ticlopidine) A - 23187

가 , 가 .

/ vWF 가 .

vWF IIb/IIIa

c7E3, KGD - RGDF -

n)	65 kD	(US 5,587,360, WO 92/07005)	(Munro, R., et al., Blood Coagulation and Fibrinolysis, 1991, 2, 179 - 184)	(Cali)
			(<i>Haementeria officinalis</i>)	16
	kD (LAPP)	(US 5,324,715).		

r L.W. et al.; Arterioscler. Thromb., 1993, 13, 1593 - 1601 Connolly T.M. et al.; Thromb. Haemostas., 1993, 69, 589).
 (soft tick, Ornithodoros moubata) (Waxman, L. et al.; J. Biol. Chem., 1993, 268, 5445 - 49) (Moubatin).
 가 Noeske - Jungblut C. et al. WO 9309137 . S
 mith et al. 50 kDa , 19 kDa (Tri
 atoma pallidipennis) .

(ADP, (pallidipin) A2 19 kDa U46619, ATP

가 , . Gan et al. GP IIa/IIIb
(Echistatin) (J. Biol. Chem, 1988, 263, 19827 - 32).

가 , vWF - 가 ,

가

가

vWF

(Saratin)

(RP - HPLC)

() , (2)
 ± 1 kDa 103

IEF - PAGE

pH 3.7 $\pm$ 0.5 ,

SDS - PAGE

(Electrospray mass spectrometry) , 3
 12061

6

가

2

LAPP

DNA

cDNA

1

1 - 63

21

64 - 372

103

2

(open reading frame)

가 , 가 (tag) , , E. coli , , E. coli 가 . pelB (E. coli) (5 mg/ ℓ) (S. cerevisiae) (> 10 mg (cross - flow) / ℓ) . COS CHO , 750 ng/ml . , , 가 , 6, 7, 8 13 , , , 가 , N - / C - 가 , [Taylor et al., J. Mol. Biol., 1986, 188, 233] , - , , 가 , , 가 , 가

III A 1, 2 3
 , 1 - , N, N' - N -
 , " 가 "
 , 가
 가
 " "
 가
 가
 가
 1
 () 가
 0.01 100 mg/ kg, 0.1 10 mg/ kg , 1
 0.5 10 mg
 0.2 150 mg/ ℓ , 1 mg 20 mg/ ℓ
 가
 가
 가 (prothese),
 가

(PCO: Posterior capsule opacification)

. PCO

. PCO

PCO

PCO

가

가

1 10

1 1

*

a) DEAE

3

(2).

b) Mono Q HR5/5

(2).

c) Mono Q HR5/5

(2) ,

RP - HPLC

(3)

2 2

RP - HPLC

SDS - PAGE.

*

(2 3).

3 3

E. coli

(7).

4 4

(Baculo)

(8)

5 5

607).

9

(#

6 6

가 , III . 9

7 7

가 , III
9

8 8

(13)

1 1

, AZOCOLL , 가 4 ,
v 가 , : 20 $\mu\text{g}/\text{m}$
가 , Horm (Nycomed) 96 (
I Nunc) . PBS 3 , 1% BSA
가 , 200 $\mu\text{g}/\text{m}$
2 가 TBS , 가
I , 가
(Centricon)

2 2

(Munro, R. et al.; Blood Coagulation and Fibrinolysis, 1991, 2, 179 - 184) LAPP (Schaffer, L.W. et al.; Arterioscler. Thromb., 1993, 13, 1593 - 1601)

, SDS - PAGE 가 80 가
1 ,

가 , Munro. R. et al. 12% 2 가 가
(190 - 250 $\mu\text{g}/\text{ml}$) ,
가 () .

, 500 ml 가 (30 - 40)

, 가

, DEAE - Fastflow EMD - DEAE - Fractogel

. 12% 2 가 , 가 가

. 가 , DEAE - , Mono Q - RP18

. , BiaCore

. DEAE - , Mono Q - RP18 1a, b, c

BiaCore , Biacore

RP18

P - RP - , SDS - PAGE (2). , PBS , R

750 μ g/ ℓ

3 3

1 , SDS - PAGE 21 kDa

(2). 48

2

12067.9 ESI - 12061.9 , 103

6 S - S 가

SDS - PAGE ,

가, Glu Asp . IEF - PAG

E (Immobiline) , pH 3.7 $\pm$ 0.5

E. coli

3 (Coomassie) (2)

/ SDS - PAGE ,

4 4

mRNA cDNA

ex mRNA " (QIAGEN) , RNA , mRNA RNA . " Oligot

" Marathon cDNA amplification kit" (CLONTECH) cDNA

PCR . cDNA DNA

AP2 , cDNA AP1

5 5

PCR

(degenerate)

N -

01 02 cDNA 5' -
 8 N -
 01 02 (degeneracy) 가
 3' - cDNA 8 가 cDNA
 . DNA (IUPAC) , R = A G; M = A C; Y = C
 T; N = A G C T .

01 02 , AP1 AP2 , 3' - RACE PCR
 . PCR TA pCR2.1 pCR Script SK(+)
 3' - RACE PCR , 5' - , N -
 8 cDNA AP1 AP2 cDNA
 ACE PCR 3' - 5' - 5' - RACE PCR , 5' - R
 PCR

15 PCR DNA ,
 가 PCR 가
 5 (silent)
 PCR 가
 ORF 372 bp , 21 103
 . PCR
 6 6

COS

COS CHO , XhoI + XbaI pCR Script SK
 (+) pCI - neo (Promega) . pCI - n
 eo T7 T3 , G418 , COS
 CHO
 3' (C -) his - MRGS(H)₆가 5' Koz
 pNC - 31

pNC - 31 DNA COS COS PBS
 2 , 1×10^7 /ml PBS , $12\mu\text{g}$ DNA (H_2O) TE
 50 μl) 0.7 - 0.8 ml COS 가 , . 1.9 kv, 2
 5 μFD 10 , 90mm . 10% FCS D
 MEM 8 ml 가 , 3
 - MRGS(H)6
 Co, Ni Cu NTA
 7 7

E. coli

E. coli E. coli E. coli
 tet pASK75 ,E. coli (Skerra,
 A., et al., Gene, 1994, 151, 131 - 135). : XbaI HindIII (linker)
 (3). ompA , - 6 $\times$ His -
 가 .
 , 5' ClaI 3' Eco47III PCR
 , 5' - 03 3' 04 . PCR PCR II (Invitrogen)
 , 5' ClaI 3' HindIII , pASK75
 PCR omp A , PCR , His -
 5' - 05 3' - 06
 E. coli , ompA pRG72 (3) ,
 W3110 (competent) . 가 , 1
 8 8

, Gibco Life Technologies Bac - To - Bac TM

5' - 07 3' - 08 PCR

BamHI 3' KpnI PCR PCR II (Invitrogen) pFastBac pTD13 (4). pTD13, m

5' BamHI 3' KpnI Bac - To - Bac (bacmid) DH10Bac

ini - attTn7 mini - Tn7 가 lacZ

mini - attTn7 mini - prep DNA E. coli DNA

9 9

()

, Sakariassen et al. [Meth. Enz., 1988, 169, 37 - 70]

()

50 mmol/L 가 III (Sigma)

(18 mm × 18 mm) 4 PBS

((20 U/ml)) 10 37

10 (30μl), (1300s⁻¹ , 37 5).

, PBS , 0.25% 30 , -

(May - Gruenwald Giemsa) 9 (80% 가)

(Leica) 가 5 (

× 1000)

6 , (#616) , 1300 s - 1

III 48%

(#607;) 81% 7

가 , 가

10 10

가 1 , 가

가 (83 - 103, 13 - 30, 58 - 69) KLH

3 N - 3 , C - 2 가

가 , Elisa ,

가 .

11 11

Horm (20 $\mu\text{g/ml}$) 50 μl (1%) (Nymcomed) 96 (Nunc) PBS 3 , BSA 50 μl 가 , 1 1 - POD (Dako) ODB - , 490 nm 2 3

12 12

7 (His -) Horm - (Nymcomed) 11 (Pierce,) - POD (750 $\mu\text{g/l}$), (epitope mapping), ODB - " "

13 13

(pichia) (Invitrogen) (pPIC9K) 8 , PCR 5' - 09 3' - 10. (Pichia) (spheroplast) , Sal I . His ⁺ Mut ⁺ - : 28 - 30 , OD 2 - 6 , 24 0.5% 6 SDS - PAGE ELISA

(57)

1.

12,000 $\pm$ 1 kD

(H. medicinalis)

2.

1 , pH 3.7 $\pm$ 0.5

3.

1 2 , - S - S - 가 6

4.

1 , 1

5.

1 80% , 4

6.

1 5

7.

2 DNA DNA DNA , 1 5

8.

7 , 1 80% DNA

9.

6 8 DNA

10.

9

11.

10

12.

1 5 , 1 10 5 , .

13.

1 5 .

14.

1 5 가 .

15.

14 , .

16.

14 15 , , 가 .

17.

1 5 .

18.

1 5 .

19.

, 1 5 .

20.

1 5 .

21.

가 , 1 5 .

22.

12 13 1
5 .

23.

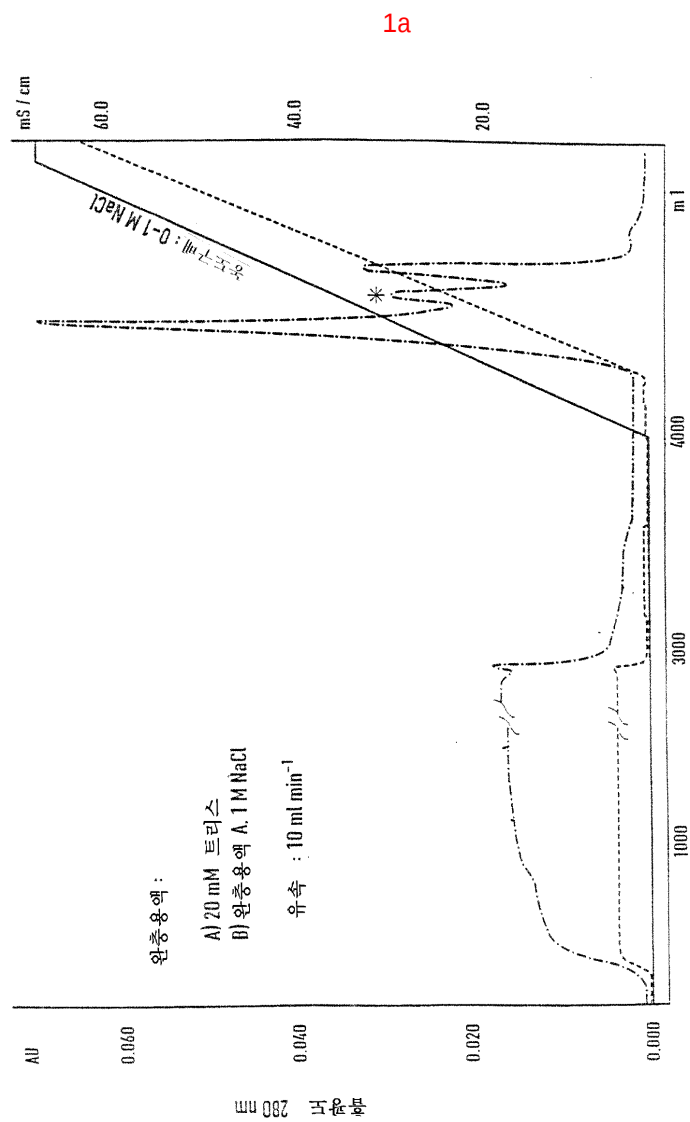
()

24.

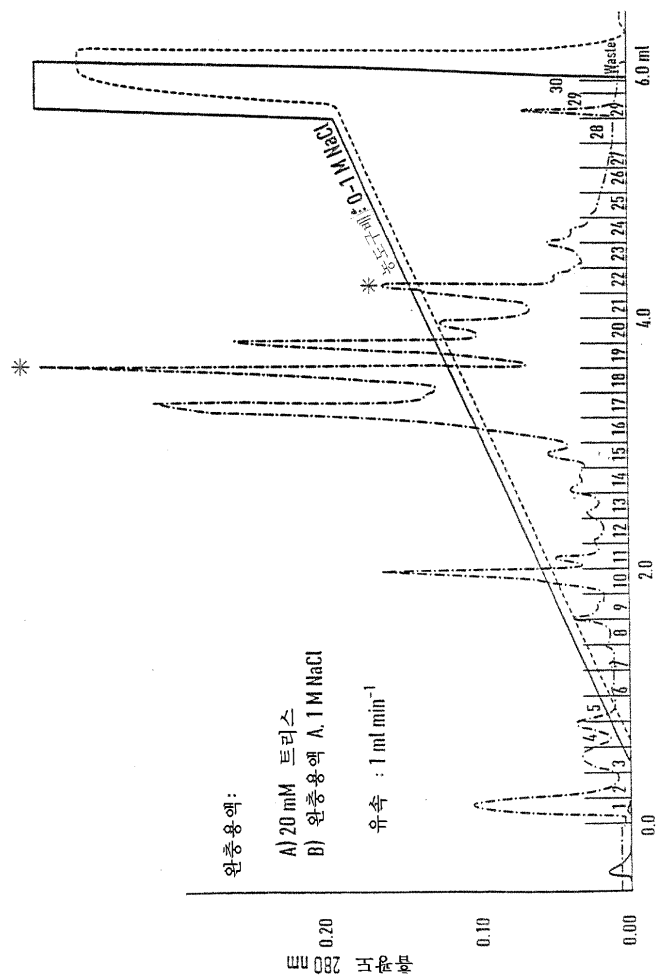
23

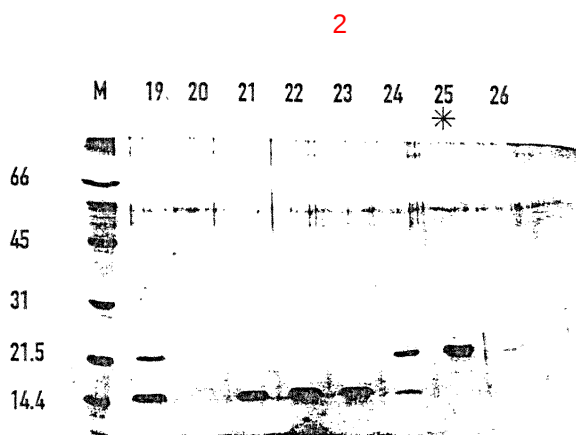
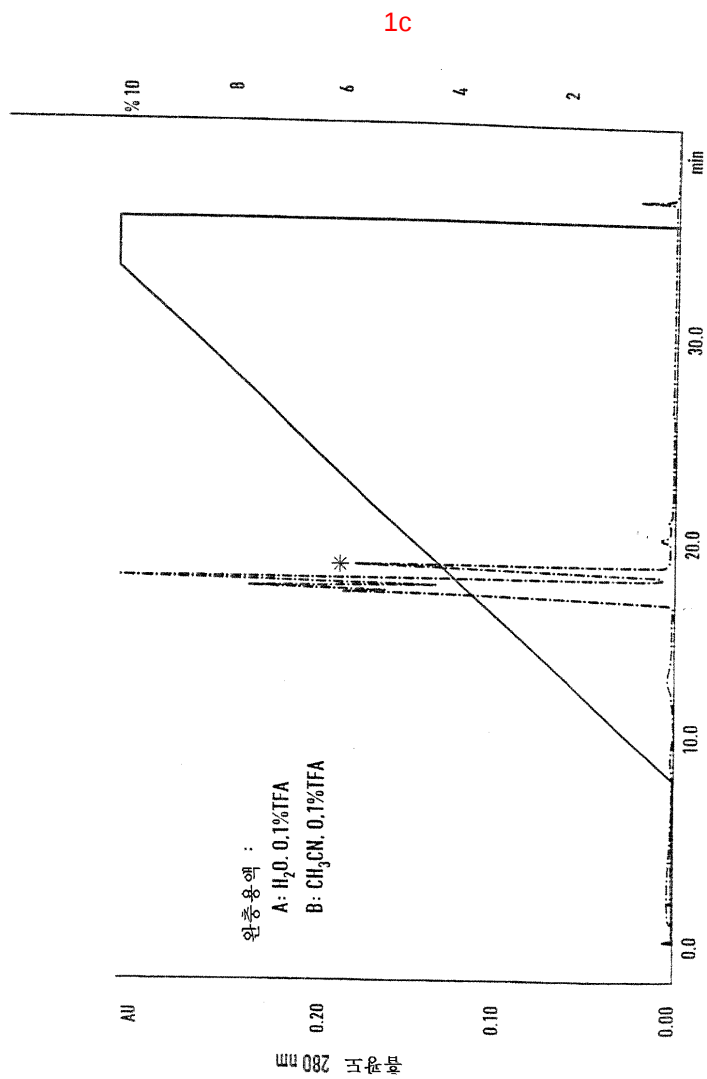
25.

23

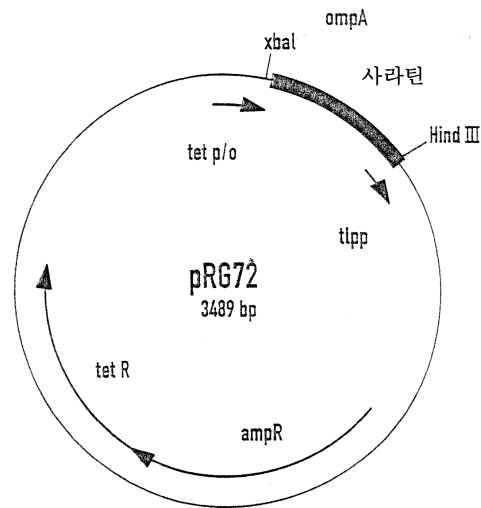


1b

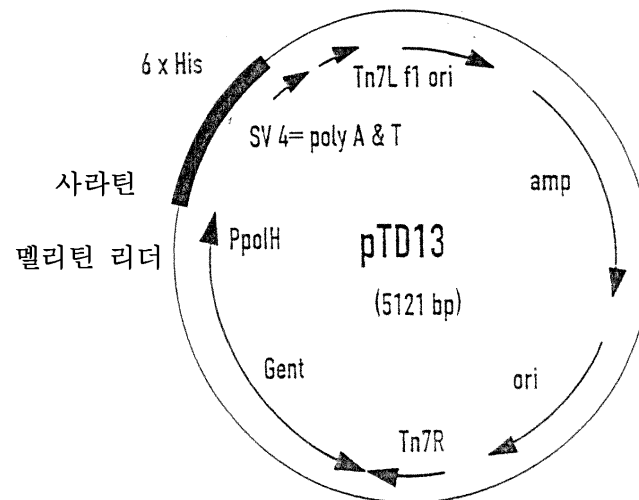




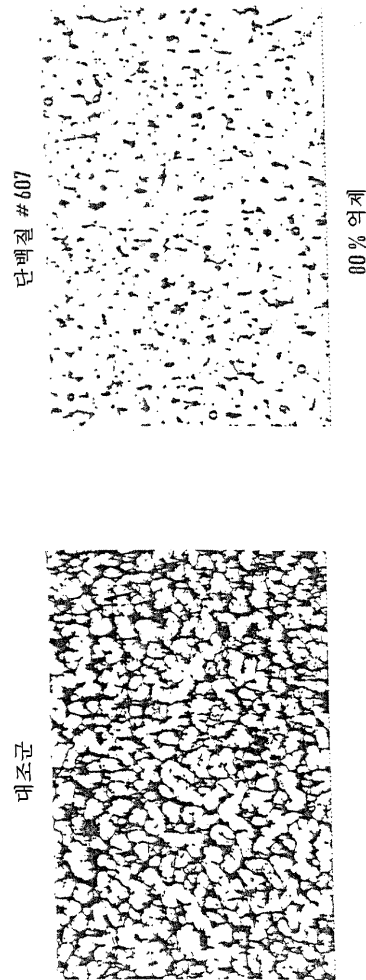
3



4

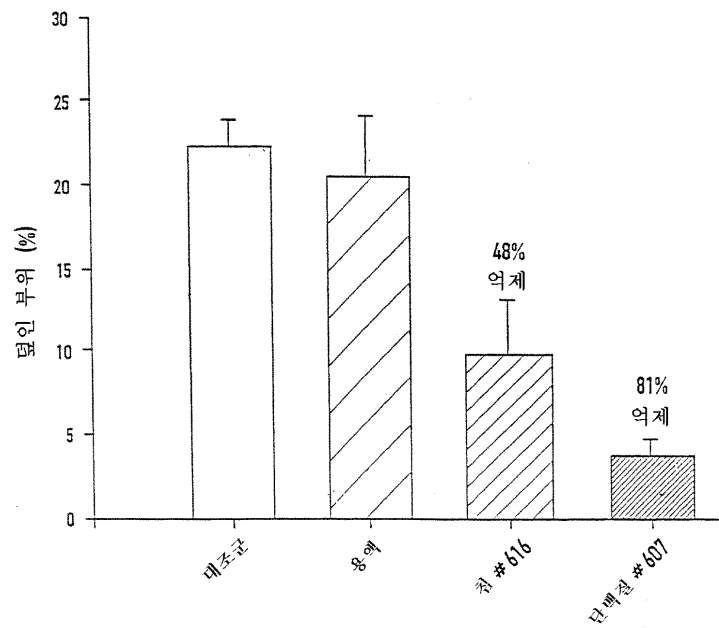


5



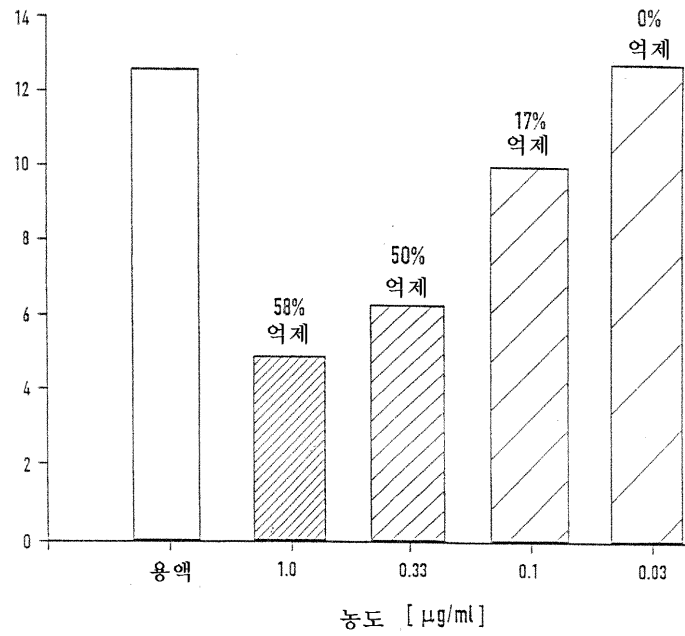
전단율 : 1300 s^{-1}
 인간 태반의 콜라겐 III 유형
 $30 \mu\text{l}$ /커버슬립

6



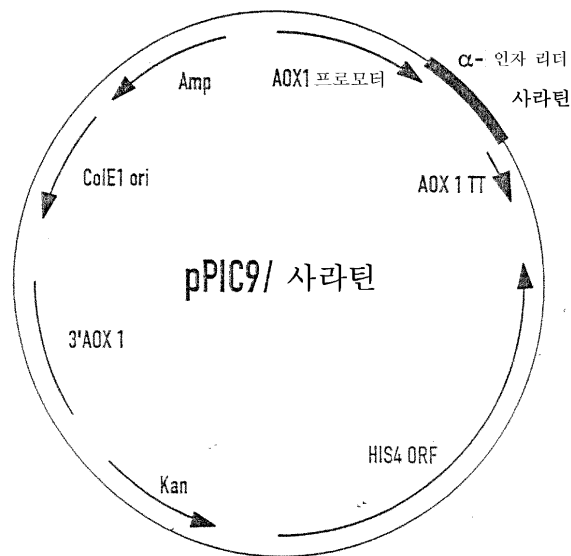
인간 태반의 콜라겐 III 유형
 침 #616 0.11 mg/ml: 커버슬립 상에 $30 \mu\text{l}$ $n=4$
 단백질 #607 0.11 mg/ml: 커버슬립 상에 $30 \mu\text{l}$ $n=2$

7



단백질 #607
인간 태반의 콜라겐 III 유형

8



<110> Merck Patent GmbH
 <120> Protein for blocking platelet adhesion
 <150> EP99105530.2
 <151> 1999-03-18
 <150> EP99109503.5
 <151> 1999-05-12

```

<160>      12
<170>      KopatentIn 1.71
<210>      1
<211>      375
<212>      DNA
<213>      Hirudo medicinalis
<220>
<221>      CDS
<222>      (64)..(372)
<400>      1
atgaagtatt tcttgatttc cttcctttgc ctcgcaagct tgctgatctc aactacttct      60
tca          gaa gaa cgt gaa gat tgt tgg acg ttt tac gcg aac aga aaa      105
              Glu Glu Arg Glu Asp Cys Trp Thr Phe Tyr Ala Asn Arg Lys
                1              5              10
tat aca gac ttc gat aaa tct ttt aag aag tcc tct gat ctt gac gaa      153
Tyr Thr Asp Phe Asp Lys Ser Phe Lys Lys Ser Ser Asp Leu Asp Glu
   15              20              25              30
tgc aaa aaa aca tgt ttc aag acg gag tac tgc tac atc gtt ttt gaa      201
Cys Lys Lys Thr Cys Phe Lys Thr Glu Tyr Cys Tyr Ile Val Phe Glu
              35              40              45
gac acg gtc aac aag gaa tgt tac tac aat gtc gtt gat ggt gaa gag      249
Asp Thr Val Asn Lys Glu Cys Tyr Tyr Asn Val Val Asp Gly Glu Glu
              50              55              60
tta gac caa gaa aaa ttt gtt gtc gac gaa aac ttc acg gaa aat tat      297
Leu Asp Gln Glu Lys Phe Val Val Asp Glu Asn Phe Thr Glu Asn Tyr
              65              70              75
ttg aca gac tgc gag ggt aaa gat gca ggt aat gcg gca ggt aca ggt      345
Leu Thr Asp Cys Glu Gly Lys Asp Ala Gly Asn Ala Ala Gly Thr Gly
              80              85              90
gac gag tca gat gaa gtt gat gaa gat          taa      375
Asp Glu Ser Asp Glu Val Asp Glu Asp
   95              100
<210>      2
<211>      103
<212>      PRT
<213>      Hirudo medicinalis
<400>      2
Glu Glu Arg Glu Asp Cys Trp Thr Phe Tyr Ala Asn Arg Lys Tyr Thr
   1              5              10              15
Asp Phe Asp Lys Ser Phe Lys Lys Ser Ser Asp Leu Asp Glu Cys Lys
              20              25              30
Lys Thr Cys Phe Lys Thr Glu Tyr Cys Tyr Ile Val Phe Glu Asp Thr
              35              40              45
Val Asn Lys Glu Cys Tyr Tyr Asn Val Val Asp Gly Glu Glu Leu Asp
              50              55              60
Gln Glu Lys Phe Val Val Asp Glu Asn Phe Thr Glu Asn Tyr Leu Thr
   65              70              75              80
Asp Cys Glu Gly Lys Asp Ala Gly Asn Ala Ala Gly Thr Gly Asp Glu
              85              90              95
Ser Asp Glu Val Asp Glu Asp

```

```

100
<210>      3
<211>      23
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence:degenerate primer01
<400>      3
gargarmgng argaytggtg gac                                     23
<210>      4
<211>      23
<212>      DNA
<213>      Artificial Sequence
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<223>      Description of Artificial Sequence:degenerate primer02
<400>      4
gargarmgng argaytgctg gac                                     23
<210>      5
<211>      24
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer03
<400>      5
gcatcgatgg aagaacgtga agac                                    24
<210>      6
<211>      23
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer04
<400>      6
tagcgctttt gacgtcgtcg tca                                    23
<210>      7
<211>      26
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer05
<400>      7
gaagaatgca aggatgagga ttattg                                   26
<210>      8
<211>      25
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer06
<400>      8
aagcttctag tcttcgtcaa cttcg                                   25
<210>      9

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```

<211>      94
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer07
<400>      9
cggatccatg aaattcttag tcaacgttgc cttgttttt atggtcgtat acatttctta      60
catctatgcg gaagaacgtg aagattgttg gact                                     94
<210>      10
<211>      24
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer08
<400>      10
ggtacctcac atatcttcat caac                                             24
<210>      11
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<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer09
<400>      11
gcatgcggcc gcctaattctt catcaacttc                                     30
<210>      12
<211>      27
<212>      DNA
<213>      Artificial Sequence
<220>
<223>      Description of Artificial Sequence: primer10
<400>      12
gcatgaattc gaagaacgtg aagattg                                         27

```