

가 , VEGF 가 [Laboratory Investigation, 71:615 (1995)].

VEGF () (vasculogenesis), (angiogenesis) [Ferrara et al., Endocrinol. Res., 18:4 - 25 (1997)].

VEGF RNA (121 , 165 , 189 206) . VEGF₁₂₁ 가 ; VEGF 가 () VEGF Arg₁₁₀ - Ala₁₁₁ VEGF (1 - 110) (: 4.6.1 3.2E3.1.1) VEGF₁₆₅ 가 FLT - 1 KDR .

VEGF KDR () FLT - 1 (FMS -) 2 가 가 , VEGF가 VEGF 가 [Malavaud et al., Cardiovascular Research, 36:276 - 281 (1997)]. KDR VEGF - VEGF [Waltenberger et al., J. Biol. Chem., 269:26988 - 26995 (1994)]. , FLT - 1 ()

VEGF [Weisman et al., Cell, 28:695 - 704 (1997); Muller et al., Proc. Natl. Acad. Sci., 94:7192 - 7197 (1997); Muller et al., Structure, 5:1325 - 1338 (1997); Fuh et al., J. Biol. Chem., 273:11197 - 11204 (1998)]. KDR VEGF₈₂ (Arg R) , 84 (Lys K) 86 (His H) VEGF FLT - 1 VEGF₆₃ (Asp D) , 64 (Glu E) 67 (Glu E) VEGF VEGF KDR [Keyt et al., J. Biol. Chem., 271:5638 - 5646 (1996)]. VEGF , VEGF가 2 KDR 가 VEGF , 2 " (hot spot)" [Muller et al.,] . 2 2 (TGF -) (PDGF) 3 - 가 -

VEGF - 가 PIGF VEGF , KDR F PDGF - 53% . PIGF Flt - 1 , PIGF

[Maglione et al., Proc. Natl. Acad. Sci., 88:9267 - 9271 (1991); Park et al., J. Biol. Chem., 269:25646 - 25654 (1994); Sawano et al., Cell Growth & Differentiation, 7:213 - 221 (1996); Landgren et al., Oncogene, 16:359 - 367 (1998)].

, 가 (Ogawa) VEGF 25% (VEGF - E)
 VEGF - E Orf (NZ - 7) 가
 , VEGF - E가 (sinusoidal)
 VEGF 가 KDR
 FLT - 1 125 | - VEGF VEGF - E 가 VE
 GF - E가 FLT - 1 KDR [Ogawa et al., J. Biological C
 hem., 273:31273 - 31281 (1998)].

[Meyer et al., EMBO J., 18:363 - 374 (1999)] , VEGF - E VEGF
 VEGF - E Orf D1701 , VEGF -
 E , VEGF
 F - E가 (Meyer) VEGF - E
 가 FLT - 1 KDR
 [Wise et al., Proc. Natl. Acad. Sci., 96:3071 - 3076 (1999)].

[Olofsson et al., Proc. Natl. Acad. Sci., 95:11709 - 11714 (1998)] " VEGF - B"
 FLT - 1 가 VEGF - B Asp63, Asp64 Glu67 가
 VEGF - B
 , FLT - 1

(VEGF) , 17 25 /
 63 65 VEGF
 , (VEGF) 43, 46, 79 83
 VEGF , 43, 46, 79 83
 VEGF 가 (VEGF) KDR
 FLT - 1 , 가 KDR FLT - 1

(VEGF) KDR
 VEGF VEGF
 ()

, VEGF 17 25 VEGF 17 25
 VEGF F17I, M18E, Y21L, Y21F, Q22R, Q22K, Q22E, Y
 25S Y25I가 , VEGF 가 VEGF 18 / 21
 , 18 가
 / 21 가

, VEGF 63 66
 VEGF , VEGF 63 66
 D63S, G65M, G65A, L66R L66T가
 VEGF VEGF 63, 65 / 66 , 65 가
 63 / 66 가
 가 VEGF VEGF 63, 65 / 66 (,)
 / VEGF 17, 18, 21, 22 / 25
 , VEGF VEGF 18 / 21
 (, 18 / 21) , VEGF 63,
 65 / 66 (, 63 , 65
 / 66) . 가 VEGF
 : M18E, Y21L, Q22R, Y25S; D63S, G65M, L66R; M18E,
 D63S, G65M, L66R; Y21L, D63S, G65M, L66R.

, VEGF 가 VEGF165
 VEGF VEGF 가 2
 가 , FLT - 1 VEGF ,
 VEGF 43, 46, 79 83 VEGF VEGF 43, 46, 79 / 83
 (,) , FLT - 1 VE
 GF 가 VEGF 43, 46, 79 / 83
 가 , FLT - 1 VEGF 가 : I43A, I46A, Q79
 A I83A.

, VEGF VEGF
 VEGF , VEGF
 가 , VEGF
 가 , , , ,
 VEGF
 () , VEGF KDR / FLT - 1 VEGF

VEGF

- 1a 1b 165 (" ") VEGF
- 2 VEGF (8 - 109) ELISA
- 3 VEGF (8 - 109) KIRA
- 4 VEGF (8 - 109) HUVEC
- 5 , KDR NIH3T3 ¹²⁵I - VEGF (1 - 165)
 VEGF (" WT VEGF"), LK - VRB - 2s* (KDR) Flt - 1 - sel (Flt - 1
) KDR 7
 , 15% 가
- 6 , FLT - 1 NIH3T3 ¹²⁵I - VEGF (1 - 165)
 VEGF (" WT VEGF"), LK - VRB - 2s* (KDR) Flt - 1 - sel (Flt - 1
) Flt - 1 7
 , 15% 가
- 7 VEGF 8
 ELISA VEGF VEGF (1 - 109)
_{IC₅₀} _{IC₅₀} IC₅₀ Flt - 1
 VEGF (1 - 109) KDR 5
 , 4 (*)가
 50% 가 , 2 가
- 8a Flt - 1 (" Flt - 1 - sel")가 470 KDR
 - (RIA) VEGF (" WT VEGF") 가
 , 15% 가
- 8b Flt - 1 (" Flt - 1 - sel")가 VEGF (" WT VEGF") FLT -
 1 - (RIA) 가
 , 15% 가
- 9 KDR VEGF (" WT VEGF") Flt - 1 (" Flt - 1 - sel")
 KIRA
- 10 HUVEC VEGF (" WT VEGF") Flt - 1 (" Flt - 1 - sel")
 HUVEC 3 가 10
 20%
- 11 ASMC MMP - 9 VEGF, LK - VRB - 2* (KDR) ,
 Flt - sel (Flt) PIGF (zymography)
 , 2가

12a 12b VEGF (" WT - VEGF"), Flt - sel (Flt) KDR (" KDR - s
 el") MAP (Western) .
 10

13a 13b PLC - PI3' - VEGF (" wt" " VEGF"), KDR
 (" KDR - sel"), Flt (" Flt - sel") KDR .
 11

14a 14b (Boyden) HUVEC .
 14a VEGF (" wt"), Flt - (" Flt - sel") KDR (" KDR - sel")
 HUVEC . 14b PI3' - (" LY") 가가 VEG
 F (" VEGF") HUVEC . 3 ,

15a 15b . 15a ,
 VEGF (" VEGF"), KDR Flt -
 . 15b , VEGF (" VEGF"), KDR (" KDR - sel"), Flt
 (" Flt - sel") PIGF .

A.

" VEGF" " VEGF"
 [Leung et al., Science, 246:1306 (1989); and Houck et al., Mol. Endocrin., 5:1806 (1991)] ()
 1a 1b) , 165 . " VEGF" " VEGF" , 121 , 189 20
 6 . " VEGF" " VEGF" , 165
 8 109 1 109
 VEGF , " VEGF (8 - 109)" , " VEGF (1 - 109)" " VEGF
 165 VEGF (1 - 165)" . " " VEGF
 VEGF 가 . , VEGF 17 ()
 VEGF 17 () . VEGF , VEGF
 KDR FLT - 1 .

" VEGF" " VEGF
 KDR FLT - 1 VEGF VEGF .
 , KDR VEGF VEGF 17 25
 / 63 66
 () . KDR VEGF
 가 , KDR VEGF () , KDR
 , VEGF FLT - 1
 (<) , FLT - 1 . KDR VEGF
 가 VEGF (가) , FLT - 1
 VEGF 가 VEGF (가) , V
 EGF 가 , KDR " " . KDR
 VEGF (VEGF) FLT - 1 가 10
 , (VEGF) FLT - 1 가 100 .

VEGF / BIAcore KDR VEGF KIRA ((KDR VEGF HUVEC KDR , ELISA, RIA KDR) 가 . KDR VEGF () HUVEC KDR) 가 . KDR

79 83 , FLT - 1 VEGF VEGF 43, 46, GF (), 가 , FLT - 1 FLT - 1 VEGF (), FLT - 1 VEGF VEGF (<), KDR VEGF (가), KDR VEGF 가 VEGF () VEGF 가 VEGF () KDR " 가 10 , VEGF FLT - 1 VEGF (VEGF) KDR 가 100 가 , ELISA, RIA / BIAcore VEGF VEGF (Leung et al.

and Houck et al.) :

- Asp D Ile I
- Thr T Leu L
- Ser S Tyr Y
- Glu E Phe F
- Pro P His H
- Gly G Lys K
- Ala A Arg R
- Cys C Trp W
- Val V Gln Q
- Met M Asn N

" " . ,

가 " "

B.

1. VEGF

VEGF [Leung et al. VEGF DNA Houck et al.,] ,
 , 2 mRNA DNA [EP 75,444
 A].

VEGF VEGF DNA DNA - DNA

가 , 가 , DNA

VEGF

VEGF 1

, 1 30 , 1 10 ,

1 - / VEGF -

) 1 10 , 1 5 (, VEGF
) , N- (

가 VEGF VEGF 가 가
 1

[1]

Ala (A)	gly; ser
Arg (R)	lys
Asn (N)	gln; his
Asp (D)	glu
Cys (C)	ser
Gln (Q)	asn
Glu (E)	asp
Gly (G)	ala; pro
His (H)	asn; gln
Ile (I)	leu; val
Leu (L)	ile; val
Lys (K)	arg; gln; glu
Met (M)	leu; tyr; ile
Phe (F)	met; leu; tyr
Ser (S)	thr
Thr (T)	ser
Trp (W)	tyr
Tyr (Y)	trp; phe
Val (V)	ile; leu

1 (a)

(c) VEGF 가 (b) 가 (a) / (P) ; (b) ; (c) ; (d) ; (e) ; (f)

가 VEGF KDR FLT - 1 VEGF VEGF VEGF ELISA, RIA, / BIAcore KIRA (HUVEC) VEGF) 가 (

VEGF DNA, cDNA, DNA, 3' - / 5' - VEGF DNA

DNA (a) VEGF VEGF 가 VEGF ; (b) VEGF (가 100) ; (c) cDNA DNA

가 DNA VEGF cDNA

DNA 가 가

" " CaPO₄ 가

" " DNA가 DN [Cohen, Proc. Natl. Acad. Sci. (USA), 69:2110 (1972); and Mandel et al., J. Mol. Biol., 53:154 (1970)] 가,

er Eb, Virology, 52: 456 - 457 (1978)] [Graham and van der Eb, Virology, 52: 456 - 457 (1978)] (Axel) 4,399,216 (1983. 8. 16.) [Van Solingen et al., J. Bact., 130: 946 (1977); and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76: 3829 (1979)] , DNA

(E. coli) K12 MM 294 (ATCC 31,446)가 DNA B X1776 (ATCC 31,537) 가

TCC 27,325), K5772 (ATCC 53,635) SR101; W3110 (F -, -, , A (Bacillus subtilis) ; (Salmonella typhimurium) (Serratia marcescens) (pseudomonas)

가

(marking)

p

BR322

[Bolivar et al.,Gene,2:95 (1977)]. pBR322

pBR322

DNA 가

()

[Chang et al.,Nature,375:615 (1978); Itakura et al.,Science,198:1056 (1977); Goeddel et al.,Nature,281:544 (1979)], (trp) [Goeddel et al.,Nucleic Acids Res.,8:4057 (1980); EPO A ppl. Publ. No. 0036,776] 가 가

[Siebenlist et al.,Cell,20:269 (1980)].

(Saccharom

yces cerevisiae)

가

가

가 YRp7 [, Stinchcomb et al.,Nature,282:39 (1979); Kingsman et al.,Gene,7:141 (1979); Tschemper et al.,Gene,10:157 (1980)]

trp1 ATCC 44,076 PEP4 - 1 [Jones,Genetics,85:12 (1977)]. trp1 가

3 -

[Hitzeman et al.,J. Biol.

Chem.,255:2073 (1980)];

- 3 -

- 6 -

, 3 -

[Hess et al.,J. Adv. Enzyme Reg.,7:149 (1968); Holland et al.,Biochemistry,17:4900 (1978)]가

3' mRNA

가

2,

C,

- 3 -

가

가

()

[Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)].

VERO HeLa , (CHO) , W138, BHK, COS - 7, 293 MDCK

; RNA ; ;

2 가

40 (SV40)

. SV40

가

가 SV40

978)].

SV40

HindIII

[Fiers et al.,Nature,273:113 (1

BglII

250 - bp

. 가 ,

가

SV40 (: , , VSV, BPV)

가

, 가

가 . 2 , (MTX) (DHFR)

VEGF DHFR

DNA DHFR

DHFR

, DHFR

DHFR

[Urlaub and Chasin, Proc. Natl. Acad. Sci. (USA), 77:4216 (1980)]

, DHFR (CHO)

, MTX 가

DHFR

DHFR

, DHFR - , MTX 가

. MTX 가 CHO CHO - K1 (ATCC CCL 61)

D

NA

10

l ()

15

15

[Goeddel et al., Nucleic Acids Res., 8:4057 (1980)]

6%

K12

294 (ATCC 31,446)

[Messing et al., Nucleic Acids Res., 9:309 (1981)]

[Maxam et al., Methods of Enzymology, 65:499 (1980)] / DNA

DNA

(MTX)

20,000

500,000 nM

, DHFR

DHF

R- , DHFR

, DHFR 가

, MTX

2. VEGF

VEGF

가

() , () ,

, ; 3- ; -2- ; - (5-) ; ; N- ; 2- ; 4- ; -7- ; -2- -1,3- ; p-

pH 5.5 7.0 pH 6.0 0.1M

; O- ; 2,4- ; ; ; ;

1 , , 2,3- , 1,2-

가 pKa

O- T 125 | 131 | 3-

2- ((4-))) (R' - N - C - N - R'), 1- -3- (4- -4,4-) -3- (가 ,

VEGF

가 1,1- () -2- , , N-

4- , [3,3' -

(-1,8-) -3- [(p-)] , - N-

가

가

3,969,287 ; 3,691,016 ; 4,195,128 ; 4,247,

642 ; 4,229,537 ; 4,330,440

가

[Creighton, Proteins: Structure and Molecular Properties 79 - 86 (W.H. Freeman & Co., San Francisco (1983)); N-

가 ; C-

가 VEGF VEGF (VEGF)

VEGF N - 가 VEGF

N - X - (, X) [Struck and Lennarz,in The Biochemistry of Glycoproteins and Proteoglycans35 (Lennarz, ed., Plenum Press (1980)), Marshall,Biochems. Soc. Symp.,40:17 (1974); and Winzler,in Hormonal Proteins and Peptides1 - 15 (Li, ed., Academic Press, New York (1973)).

O - 가 , O - ,가 N - , 5 - 5 -

[The Plasma Proteins: Structure, Function and Genetic Control271 - 315 (Putnam, ed., 2nd edition, Academic Press, New York (1984))

3가 O - 가 [Aplin and Wriston inCRC Crit. Rev. Biochem.259 - 306 (1981)]

O - N - () (a) , (b) , (c) , (d) , (e) (f) PCT WO 87/05330 (1987. 9. 11)

[Tanner and Lehle,Biochim. Biophys. Acta,906 (1):81 - 99 (1987) and Kukuruzinska et al.,Annu. Rev. Biochem.,56:915 - 944 (1987)]

VEGF 가 [Walter et al.,Laboratory Investigation,74:546 (1996)], VEGF

3.

VEGF KDR FLT - 1 V EGF 가 KDR 가 , Flt - 1 KDR VEGF 가 , Flt - 1

(: , , ,) ,

. VEGF

, VEGF

가

, VEGF

, VEGF
, VEGF

가

가 , 가
가 , 가 VEGF

가

VEGF

가

0.35

Li, N

a, K, Cs

가

가

가

가

VEGF

USP

가

가

VEGF
3%

300

1000 mg

2

5%

400

600

1

500

0.1 ng/cc , VEGF VEGF 가 ,

(: VEGF) aFGF, bFGF, HGF, PDGF, IGF, NGF, TGF -) VEGF VEGF

KDR VEGF VEGF VEGF 가 가 가 가 가 ³H, ¹⁴C, ³²P, ³⁵S ¹²⁵I ; VEGF ;

VEGF KDR Flt - 1 VEGF VEGF KDR KDR Flt - 1 Flt - 1 VEGF KDR F

4.

가 가 VEGF 가 () VE GF 2 가 (, , ,) 가 2 3

1

KDR - VEGF

KDR - VEGF (1 - 109) 가 , Flt - 1 KDR

Inc.) . Nsi I/Xba I VEGF 1 - 109 109 , VEGF 1 - 109 cDNA PCR phGHam - g3 (Genentech, Inc.) VEGF 1 - 109 cDNA pB2105 (Genentech, 249 406 gIII C -

T C , S=C , VEGF 1 - 109 (G) 18, 21, 22 25 () 가 NNS (, N=G, A, 17 40% (3가 10% 70%) 가

NNS

L - 528: CAC GAA GTG AAGTTCNNS GAT GTC NNS NNS CGC AGC NNS TGC CAT CCA ATC GAG (1)

L - 530: GGG GGC TGC TGC AAT NNS GAG NNS NNS GAG TGT GTG CCC ACT (2).

40% , VEGF 1 - 109 63, 65 66 가 64

46 , VEGF (1 - 109) 47 48 가 43 50% 가 63, 65 66 가 64 50% 가

KDR , 5 , 4 50% " 가 " , 2 가 " (*)가 " (7)

가 DNA

가 DNA [Kunkel et al., Meth. Enzym. 204:125 - 139 (1991)]

DNA (CCC - DNA)

5' - (500 mM - HCl, 100 mM MgCl₂, pH 7.5), 2 μl 10 mM ATP 2 μg, 1 μl 100 mM DTT 2 μl 10x TM 가 20 μl가 가 T4 20 (Weiss)) 가 37 1

5' - (dut - /ung - CJ - 236 DNA) . 1 μg 가 DNA , 0.12 μg 2.5 μl 10x TM (500 mM - HCl, 100 mM MgCl₂, pH 7.5) 가 25 μl가 가 DNA 가 1:100 3:1 가 20 5 90 2 50 3

5' - CCC - DNA : 1 μl 10 mM ATP, 1 μl 25 mM dNTPs, 1.5 μl 100 mM DTT, 3 T 가 3 T4 DNA , 20 3 DNA 15 μl

XL1 - (Stratagene, LaJolla, CA)

DNA 가 XL1 - 100 μl DNA 0.2 - cm : 2.5 kV , 200 ohms 25 mF (Gene Pulser (Bio - rad, Hercules, CA)) , SOC 1 Mℓ (5 g - , 20 g - , 0.5 g NaCl, 0.2 g KCl; 1 가 NaOH pH 7.0 ; ; 2 M MgCl₂ 5 mL 1 M 가) 가 37 30

1 가 NaOH pH 7.0 ; 2YT (10 g - , 16 g - , 5 g NaCl; , 25 Mℓ 2YT, 25 mg/Mℓ) (50 μg/Mℓ) 250 Mℓ , M13 - VCS (10¹⁰ pfu/mL) (Stratagene, LaJolla, CA) 37

(Sorvall) GSA (16000 g) 10 krpm, 2 10 1/5 PEG/NaCl (200 g/L PEG - 8000, 146 g/L NaCl;) 가 /PEG - NaCl 5

PBT (PBS, 0.2% BSA, 0.1% 20) 1/20 , SS - 34 (27000 g) 15 krpm, 2 5

가 KDR () Flt - 1
 VEGF (1 - 109)
 - gIII

69 (1996)] [H. Jin, J. Clin. Invest., 98: 9
 KDR (1 - 3)

entech, Inc.) KDR (1 - 3) (100 nM) Flt - 1 (1 - 3) (Gen
 (Genentech, South San Francisco, California)
 (Nalge Nunc International, Rochester, New York)
 80 μ l (pH 9.6 50 mM) KDR (1 - 3) (2 - 5 μ g/Ml) 4

PBS 0.2% BSA 200 μ l 1

PT (PBS, 0.05% 20) 8 , PBT (PBS, 0.
 2% BSA, 0.1% 20) (10¹² /Ml) 100 μ l
 가 Flt (1 - 3) 가 . 2

PT (PBS, 0.05% 20) 10 Flt - 1
 pH 2 5 0.2 mM 100 μ l KDR -
 . KDR , pH 8.0 1.0 M
 - HCl

0 < 10) 가 37 30 XL1 - (OD₆₀
 2YT/amp (2YT 50 mg/Ml) .
 R (1 - 3) . KD

37 10 2YT/amp/VCS (2YT 50 mg/Ml) 10¹⁰ pfu/Ml M13 - VCS)

(100 nM) Flt - 1 (1 - 3) KDR (1 - 3)
 , Flt - 1 - 가 (5 6) KDR

KDR (1 - 3)
 . 1 KDR (1 - 3)
 가 Flt - 1 (1 - 3) . 가 ,

ELISA

VEGF 1 - 109 - gIII KDR (1 - 3) [Mul
 ler et al., PNAS, 94: 7192 (1997)] ELISA ELISA
 (Maxisorp, Nunc - Immunoplate, Nalge Nunc International, Rochester, New York) pH 9.6
 50 mM KDR (1 - 3) Flt - 1 (1 - 3) (5 $\mu\text{g}/\text{Ml}$) 4
 0.5% BSA (KDR (1 - 3)) Flt - 1 (1
 - 3)) VEGF 1 - 109 (PBS, 0.5% 20, 0.5
 % BSA) 100 μl 가 , ,
 - M13 (Pharmacia Biotech, Piscataway, NJ) (EC₅₀)

cDNA Flt - 1 (1 - 3) VEGF 1 - 109

VEGF 1 - 109

VEGF 1 - 109 (27c7)
 [Yihai et al., J. Biol. Chem., 271:3154 - 3162 (1996)]
 pH 6 6 M HCl + 1 mM , pH 8 10
 20 mM - HCl 2 mM 0.5 mM 2 M 10
 . 4 20 mM - HCl (pH 8) 20
 (Pharmacia HiTrap Q, 1 Ml) (Pharmacia Biotech, Piscataway, NJ)
 SDS - PAGE

2 VEGF ,
 (*) VEGF
 . " s" (, LK - VRB - 1s) VEGF 1 - 109 VEGF
 VEGF 1 - 165 . " f" (, LK - VRB - 1f) VEG
 F , " M18E" VEGF 18 가 (,
 (Leung et al., Houck et al.,) VEGF VEGF
 GF) " (M) (E) VE
 " GAG" , 2 , M18E

VEGF

LK - VRB - 1s*	M18E/Y21L/Q22R/Y25S	GAG/CTC/CGG/AGC
LK - VRB - 2s*	D63S/G65M/L66R	AGC/ATG/CGC
LK - VRB - 3s	F17I/M18E/Y21F/Q22K/Y25S	ATT/GAG/TTC/AAG/AGC
LK - VRB - 4s	F17I/M18E/Y21F/Q22E/Y25I	ATC/GAG/TTC/GAG/CAC
LK - VRB - 5s	D63S/L66R	AAG/CAG
LK - VRB - 6s	D63S/G65A/L66T	AAG/GGC/ATG
LK - VRB - 7s*	M18E/D63S/G65M/L66R	GAG/AGC/ATG/CGC
LK - VRB - 8s*	Y21L/D63S/G65M/L66R	CTC/AGC/ATG/CGC
LK - VRB - 9s	Q22R/D63S/G65M/L66R	CGG/AGC/ATG/CGC
LK - VRB - 10s	Y25S/D63S/G65M/L66R	AGC/AGC/ATG/CGC
LK - VRB - 11s	M18E/Y21L/D63S/G65M/L66R	GAG/CTC/AGC/ATG/CGC
LK - VRB - 12s	M18E/Q22R/D63S/G65M/L66R	GAG/CGG/AGC/ATG/CGC
LK - VRB - 13s	M18E/Y25S/D63S/G65M/L66R	GAG/AGC/AGC/ATG/CGC
LK - VRB - 14s	Y21L/Q22R/D63S/G65M/L66R	CTC/CGG/AGC/ATG/CGC
LK - VRB - 15s	Y21L/Y25S/D63S/G65M/L66R	CTC/AGC/AGC/ATG/CGC
LK - VRB - 16s	Q22R/Y25S/D63S/G65M/L66R	CGG/AGC/AGC/ATG/CGC
LK - VRB - 17s	M18E/Y21L/Q22R/D63S/G65M/L66R	GAG/CTC/GAG/AGC/ATG/CGC
LK - VRB - 18s	M18E/Q22R/Y25S/D63S/G65M/L66R	GAG/CGG/AGC/AGC/ATG/CGC
LK - VRB - 19s	M18E/Q22R/Y25S/D63S/G65M/L66R	GAG/CGG/AGC/AGC/ATG/CGC
LK - VRB - 20s	Y21L/Q22R/Y25S/D63S/G65M/L66R	CTC/CGG/AGC/AGC/ATG/CGC
LK - VRB - 21s	D63S/M18E/Y21L/Q22R/Y25S	TCC/GAG/CTC/CGG/AGC
LK - VRB - 22s	G65M/M18E/Y21L/Q22R/Y25S	ATG/GAG/CTC/CGG/AGC
LK - VRB - 23s	L66R/M18E/Y21L/Q22R/Y25S	AGG/GAG/CTC/CGG/AGC
LK - VRB - 24s	D63S/G65M/M18E/Y21L/Q22R/Y25S	TCC/ATG/GAG/CTC/CGG/AGC
LK - VRB - 25s	D63S/L66R/M18E/Y21L/Q22R/Y25S	TCC/AGG/GAG/CTC/CGG/AGC
LK - VRB - 26s	G65M/L66R/M18E/Y21L/Q22R/Y25S	ATG/AGG/GAG/CTC/CGG/AGC
LK - VRB - 27s	M18E/Y21L/Q22R/Y25S/D63S/G65M/L66R	GAG/CTC/CGG/AGC/AGC/ATG/CGC
LK - VRB - 1f	M18E/Y21L/Q22R/Y25S	GAG/CTC/CGG/AGC
LK - VRB - 2f	D63S/G65M/L66R	AGC/ATG/CGC

2

KDR VEGF

KDR VEGF (1 - 109) VEGF 165 (1) 가 . 가 KDR VEGF
2

96 - (Maxisorp, Nunc - Immunoplate, Nalge Nunc International, Rochester, New York) pH 9.6 50 mM MAKD5 (Genentech, South San Francisco, California) KDR 8 µg/ml 100 µl 4
1 (0.5% BSA, PBS 0.01%) (150 µl).

(VEGF (8 - 109), VEGF (1 - 165), VEGF (1 - 109) VEGF 165
 0.16 - 168 nM) (0.5% BSA, PBS 0.05% 20) 2
 (100 μℓ) VEGF (8 - 109) (84 nM) KDR (1 - 3) (1 μg/Mℓ)
 가 1
 (0.2 mg/Mℓ, Sigma, St. Louis, Missouri) 30
 KDR (1 - 3) VEGF
 10 3,3',5,5' - (0.2 g/ ; Kirkegaard & Perry Laboratories, Gaithersbu
 rg, Maryland) . Vmax (Molecular Devices, Menlo Park, California)
 450 nm
 4 - (KaleidaGraph, Synergy Software, Reading, Pennsylv
 ania) VEGF (8 - 109) VEGF VEGF (2).
 VEGF VEGF (1 - 109) VEGF 165 3 VEGF
 VEGF (8 - 109) 2 KDR
 3
 Flt - 1 VEGF
 Flt - 1 VEGF (8 - 109) Flt - 1
 VEGF (1 - 109) VEGF 165 (1) 가 가 VE
 GF 2
 96 - (Maxisorp, Nunc - Immunoplate, Nalge Nunc International, Rochester, Ne
 w York) pH 9.6 50 mM IgG Fc F(ab')2 (Jack
 son ImmunoResearch, West Grove, Pennsylvania) 2 μg/Mℓ 100 μℓ 4
 (PBS 0.5% 20) 3
 1 (0.5% BSA, PBS 0.01%) (150 μℓ).
 (0.5% BSA, PBS 0.05% 20) 50 ng/Mℓ Flt - IgG (Flt - Fc)
 100 μℓ 1 (PBS 0.05%
 20) 3
 VEGF (8 - 109), VEGF 165, VEGF (1 - 109) VEGF 165 (V
 0.03 - 33 nM) VEGF (8 - 109) (0.21 nM) 가
 EGF 165 (0.66 nM) (100 μℓ)
 2 (0.2 mg/Mℓ, Sigma, St. Louis, M
 issouri) 30 Flt - IgG
 VEGF 10 3,3',5,5' - (0.2 g/ ; K
 irkegaard & Perry Laboratories, Gaithersburg, Maryland) . Vmax (Molecu
 lar Devices, Menlo Park, California) 450 nm

4 - (KaleidaGraph, Synergy Software, Reading, Pennsylv
 ania) VEGF (8 - 109) VEGF
 VEGF VEGF
 VEGF (1 - 109) VEGF 165 3 VEGF
 VEGF (8 - 109) 2,000 Flt - 1 VEGF
 (, LK - VRB - 7s* LK - VRB - 8s*) 3 Flt - 1
 nM , 가 ELISA

	KDR	FLT - 1	VEGF
			KDR FLT - 1
LK - VRB - 1s*	1 nM/1		2700 nM/6000
LK - VRB - 2s*	1 nM/1		> 400 nM/> 1000
LK - VRB - 3s	1 nM/1		170 nM/400
LK - VRB - 4s	1 nM/1		100 nM/200
LK - VRB - 5s	1 nM/1		233 nM/550
LK - VRB - 6s	0.5 nM/0.5		4 nM/10
LK - VRB - 7s*	1 nM/1		/> 15000
LK - VRB - 8s*	0.5 nM/0.5		/> 21000
LK - VRB - 9s	0.5 nM/0.5		/300
LK - VRB - 10s	0.5 nM/0.5		/> 2400
LK - VRB - 11s	2 nM/2		/> 14000
LK - VRB - 12s	0.4 nM/0.4		/> 5600
LK - VRB - 13s	14 nM/14		/> 14000
LK - VRB - 14s	0.5 nM/0.5		/> 2900
LK - VRB - 15s	2 nM/2		/> 21000
LK - VRB - 16s	0.6 nM/0.6		/> 1400
LK - VRB - 17s	3 nM/3		/> 1900
LK - VRB - 18s	130 nM/130		/> 3900
LK - VRB - 19s	7 nM/7		/> 35000
LK - VRB - 20s	2 nM/2		/> 10000
LK - VRB - 21s	3 nM/3		/> 5600
LK - VRB - 22s	4 nM/4		/> 30
LK - VRB - 23s	11 nM/11		/> 8500
LK - VRB - 24s	10 nM/10		/> 18000
LK - VRB - 25s	4 nM/4		/> 12000
LK - VRB - 26s	23 nM/23		/> 25000
LK - VRB - 2f	1 nM/1		19 nM/70
VEGF (8 - 109)	1 nM/1		0.42 nM/1

4

VEGF (1 - 109) KDR
 VEGF , KDR KIRA
 가 VEGF 2 , VEGF (1 - 109)
 : LK - VRB - 1s*; LK - VRB - 2s*; LK - VRB - 3s; LK - VRB - 4s; LK - VRB - 5s; LK - VRB - 6s.

VEGF (1 - 109) (0.01 - 10 nM) N - gD 가 KDR CHO
 가 pH 7.2 0.5% - X100, 150 mM NaCl, 50 mM Hepes ,
 gD - KDR ELISA .

ELISA , 96 - (Maxisorp, Nunc - Immunoplate, Nalge Nunc International, Rochester, New
 York) pH 9.6 50 mM 3C8 (Genentech, South San Francisco, Ca
 lifornia) gD 1 µg/Mℓ 100 µℓ 4
 1 (0.5% BSA, PBS 0.01%) (150 µℓ).

(100 µℓ) 가 2 4G10 (0.05 mg
 /Mℓ) (Upstate Biotechnology, Lake Placid, New York)
 2 (0.2 mg/Mℓ,
 Sigma, St. Louis, Missouri) 1 gD - KDR
 15 - 20 3,3',5,5' - (0.2 g/ ; Kirkegaard & Perry Laborat
 ories, Gaithersburg, Maryland) . Vmax (Molecular Devices, Menlo Park, C
 alifornia) 450 nm

4 - (KaleidaGraph, Synergy Software, Reading, Pennsylv
 ania) VEGF (8 - 109) VEGF VEGF (3).
 VEGF 4 . VEGF VEGF (8 - 109)
 2

VEGF (1 - 109)	KDR
LK - VRB - 1s*	1 nM/0.5
LK - VRB - 2s*	2 nM/1
LK - VRB - 3s	2 nM/1
LK - VRB - 4s	1 nM/0.5
LK - VRB - 5s	1 nM/0.5
LK - VRB - 6s	1 nM/0.5
VEGF (8 - 109)	2 nM/1

5

VEGF (1 - 109) VEGF 165 (VEGF 165 LK - VRB - 2f)
 가 VEGF 2 (HUVEC) (Cell Systems, Kirkland, Washington) . VEGF (1 - 109) :
 LK - VRB - 1s*; LK - VRB - 2s*; LK - VRB - 8s*.

HUVEC CS - C (Cell Systems, Kirkland, Washington) FGF (5) 9
 6 - (100 μℓ 3000) 2% (Gi
 bcoBRL, Gaithersburg, MD) CS - C 5% CO₂ 가 VEGF 가
 37 24 가 VEGF 가
 150 μℓ 18
 VEGF DNA ,³H - (Amersham Life Science, Arlington Heig
 hts, IL) 0.5 μCi 가 , 24 가
 96 - (Topcount) (Packard, Meriden, Connecticut)
 (CPM)
 (4).
 VEGF 5 . VEGF VEGF (8 - 109)
 가 2 가 .

VEGF (1 - 109)

LK - VRB - 1s*	0.1 nM/0.2
LK - VRB - 2s*	0.05 nM/0.1
LK - VRB - 7s*	0.5 nM/1
LK - VRB - 8s*	0.5 nM/1
LK - VRB - 2f	0.05 nM/0.1
VEGF (8 - 109)	0.5 nM/1

6

KDR FLT - 1 VEGF RIA
 [Muller et al., PNAS, 94:7192 - 7197 (1997)] RIA
 VEGF 165 VEGF (8 - 109) , KDR FLT - 1 가 VEGF ()
 2)

[6]

	KDR	FLT - 1
VEGF 165	1 (97 pM)	1 (37 pM)
VEGF (8 - 109)	12	29
LK - VRB - 1f	8	1700
LK - VRB - 1s*	20	14,000
LK - VRB - 2f	1	2400
LK - VRB - 2s*	2	27,000

7

KDR - FLT - 1 - VEGF

LK - VRB - 2s* (1, 2) - KDR
 Flt - 1 NIH3T3 [Fuh et al., J. Biol. Chem., 273:11187 - 11204 (1998)]
 10% FBS 400 µg/Mℓ G418 (GibcoBRL) F12
 12 - 1 x 10⁶/
¹²⁵I - VEGF (1 - 109) (- T) 가 VEGF
 가 1 1% BSA 가 (HBS) . 50 pM 10
 pM VEGF (1 - 109) KDR Flt - 1 . 4 3
 0.5% BSA 가 HBS 2 . 1N NaOH 가 , VE
 GF (Isodata, ICN)

5 , LK - VRB - 2s* VEGF KDR
 , LK - VRB - 2s* Flt - 1 200 ()
 6) .

8

Flt - 1 VEGF

(Wells, Methods Enzymol., 202:390 - 411 (1991))

VEGF KDR Flt - 1
 VEGF KDR Flt - 1
 2 22 Phe
 47 Glu 64 [Muller et al., Proc. Natl. Acad. Sci., 94:7192 - 7197 (1997)].
 VEGF (1 109) [Kunkel et al., Methods Enzymol., 204:12
 5 - 139 (1991)] DNA VEG
 F 가 : Lys 16, Phe 17, Met 18, Tyr 21, Gln 22, Tyr 25, Ile 43, Ile
 46, Phe 47, Lys 48, Asp 63, Glu 64, Gly 65, Leu 66, Gln 79, Met 81, Ile 83, His 86, Gln 89, Ile 91, Lys 1
 01, Glu 103, Arg 105, Pro 106. VEGF
 [1 109: Keyt et al., J. Biol. Chem., 271:7788 - 7795 (1996); Muller et al., Proc. Natl.
 Acad. Sci., 94:7192 - 7197 (1997)].

(ELISA) KDR Flt -
 VEGF ; Wiesm
 ann et al., Cell, 91:695 - 704 (1997); Fuh et al., J. Biol. Chem., 273:11197 - 11204 (1998)]. ELISA
 , 50 mM (pH 9.6) VEGF (8 - 109) (5 µg/Mℓ) 4
 . 0.5% BSA , VEGF (100 pM) -
 (KDR(1 - 3) Flt1(1 - 3)) (0.5% 20, PBS 0.5% BSA) 100
 µℓ 가 . 1 , , 50% KDR(1 - 3) Flt(1 - 3)
 (Pharmacia) 가 .
 IC₅₀ 가 .

7 , VEGF (8 - 109) IC₅₀ , IC₅₀ 가 . VEGF (8 - 109)
 IC₅₀ Flt - 1 .
 Flt - 1 VEGF 20s 60s
 , 가 Flt - 1 - Phe 17, Tyr 21, Gln 22 Leu 66 (7).
 , Flt - 1 - KDR
 (7).

10

MAP

VEGF, KDR - VEGF Flt - VEGF 가

4 - 7 HUVEC (Cell Systems, Kirkland, WA) 10%

가 (AZO - 500) 14 0.2%

HUVEC VEGF (1 - 165) VEGF (Flt - 1

165 8 " " (1 - 109) Flt - sel

I43A/I46A/Q79A/I83A); LK - VRB - 2f (1; 2) KDR -

(165) (50 ng/Mℓ 10 ng/Mℓ) 5 VEGF (1

- 165) VEGF [Keyt et al., J. Biol. Chem., 271:5638 - 5646 (1996)]

, HUVEC 0.1 mM , 5 mM -

, 10 mM , 0.5 (Roche MB 1836145)

0.5 - 1 Mℓ RIPA , - ERK

(Promega) ERK1 ERK2

KDR VEGF LK - VRB - 2f HUVEC ERK1 ERK2

(12a). VEGF (1 - 165) Flt - 1

VEGF () ERK2 가

VEGF , Flt - 1 MAP

VEGF - , p38 MAP [Rousseau et al., Oncogene, 15:2

169 - 2177 (1997); Yu et al., J. Cell. Phys., 178:235 - 246 (1999)]. VEGF 가

, VEGF (1 - 165), Flt - 1 LK - VRB - 2f () p38

4 - 7 HUVEC (Cell Systems, Kirkland, WA) 10%

가 (AZO - 500) 14 0.2%

HUVEC VEGF (1 - 165) VEGF (Flt - sel (1

65) LK - VRB - 2f; ERK1 ERK2) (50 ng/Mℓ 10 ng/Mℓ) 5

, , 0.1 mM , 5 mM - , 10 mM

, 0.5 (Roche MB 1836145) 0.5 - 1

Mℓ RIPA . p38 MAP - p38 (

NEB) 가

12b KDR - VEGF p38

11

KDR PI 3' - PLC -

PLC - VEGF KDR [Dougher et al., *Oncogene*, 18:1619 - 1627 (1999); Cunningham et al., *Biochem. Biophys. Res. Comm.*, 240:635 - 639 (1997)] Flt - 1 [Seetharam et al., *Oncogene*, 10:135 - 147 (1995); Sawano et al., *Biochem. Biophys. Res. Comm.*, 238:487 - 491 (1997); Ito et al., *J. Biol. Chem.*, 273:23410 - 23418 (1998)] PLC -

VEGF () 1 PLC - , HUVEC
 VEGF VEGF - PLC - 가 .

4 - 7 HUVEC (Cell Systems, Kirkland, WA) 10%
 가 (AZO - 500) 14 0.2% .
 HUVEC VEGF (1 - 165) VEGF (Flt - sel (1
 65) LK - VRB - 2f; 10) (20 ng/M ℓ) 5 . , 0.
 1 mM , 5 mM - , 10 mM , 0.5
 (Roche MB 1836145) 0.5 - 1 M ℓ RIPA .
 , PLC - (Upstate Biotechnology)
 (13a) p85 PI 3' - (Transduction L
 abs (P13020) Neomarkers (MS424 - P)) PY20 E120
 H (Transduction Labs) (13b).
 A/G (Pierce) 50 mM HEPES pH 7.2, 0.1% TX - 100, 150 mM NaCl 1 mg/M ℓ 30
 . 3 가 . 50 mM
 pH 7.6, 150 mM NaCl, 1% TX - 100, 1 mM CaCl $_2$; 50 mM pH 7.6, 500 mM NaCl, 0.1% TX - 10
 0, 1 mM CaCl $_2$ 50 mM pH 7.6, 150 mM NaCl, 0.05% TX - 100, 1 mM CaCl $_2$
 . 2x . 4 - 12% - (Novex)
 가 .

13a , VEGF KDR - VEGF PLC -
 가 . Flt - 1 VEGF PLC - 가 ,
 HUVEC PLC - Flt - 1 .

PI 3' - 가 Akt [Marte e
 t al., *Trends Biochem. Sci.*, 22:355 - 358 (1997)]. VEGF
 PI - 3' Akt [Gerber et al., *J. Biol. Chem.*, 273:30366 - 30343 (199
 8)]. , PI - 3'
 [Wennstrom et al., *Curr. Biol.*, 4:385 - 393 (1994)]. , VEGF PI - 3'
 p85 가 가 . 13b , VE
 GF KDR - VEGF PI - 3'

12

VEGF
 . HUVEC

8.0 (Cell Systems) (Falcon 3097) 1 (VITROGEN, COHESION) HUVEC
 , < 8) 10% FCS 가 (4ZO - 500)
 0.1% BSA 가 EBM (, Clonetics)
 5 x 10⁴ (VEGF (1 - 165); Flt - 1 ; LK - VRB - 2f; 10
) (14a 14a)
 37 18 . LY294002 가 30
) 가 20 , VEGF 가 1 LY294002 (Biomol
) 4

- (Yo - Pro Iodide) (Molecular Probes) -

14a () HUVEC - VEGF
 HUVEC (3 ;) 가 VEGF HUVEC VEGF
 VEGF 4 - 5 가 . KDR - VEGF HUVEC 가 가
 Flt - 1 VEGF
 LY 294002 PI 3 - 가 가
 () . 14b LY 294002가 PI 3' - HUVEC 56%
 , PI 3' -
 13

[Polverini et al., Methods Enzymol., 198:440 - 450 (1991)]

가 () / (80 mg/kg) / (15 mg/kg)
 . #15
 1.5 mm (ST80017, ASSI)
 (200 ng) (VEGF (1 - 165); Flt - 1 ; LK - VRB - 2f; (
 10) PIGF (R & D Systems), (100 µg) (
 (2 mm x 2 mm)
 . 6 , FITC - 가 가
 (Image - Pro Plus)

15a , KDR - VEGF VEGF
 . Flt - 1 VEGF 가 (15a),
 . P1GF Flt - 1 VEGF 가 가
 (15b). , Flt - 1 KDR

(57)

1.	VEGF				KDR	
	VEGF	.				
2.						
1	,		가 VEGF	VEGF	17	25
				.		
3.						
1	,		가 VEGF	VEGF	63	66
				.		
4.						
1	,	가 4				4 가
		: M18E, Y21L, Q22R, Y25S	VEGF	.		
5.						
1	,	가 3				3 가
		: D63S, G65M, L66R	VEGF	.		
6.						
1	,	가 4				4 가
		: M18E, D63S, G65M, L66R	VEGF	.		
7.						
1	,	가 4				4 가
		: Y21L, D63S, G65M, L66R	VEGF	.		
8.						
1	VEGF	DNA		.		
9.						
8	,	DNA	4	VEGF		.
10.						
8	,	DNA	5	VEGF		.

11.

8 , DNA 6 VEGF .

12.

8 , DNA 7 VEGF .

13.

8 .

14.

13 .

15.

1 VEGF .

16.

15 , 가 .

17.

() 1 VEGF , KDR KDR
VEGF .

18.

KDR 1 VEGF .

19.

15 .

1a

```

1  CAGTGTGCTG GCGGCCCGGC GCGAGCCGGC CCGGCCCGG TCGGGCCTCC
-26
    GAAACC  ATG AAC TTT CTG CTG TCT TGG GTG CAT TGG AGC
           M  N  F  L  L  S  W  V  H  W  S
           -26                    -20
90  CTC GCC TTG CTG CTC TAC CTC CAC CAT GCC AAG TGG TCC CAG
-15 L  A  L  L  L  Y  L  H  H  A  K  W  S  Q
           -10
    GCT GCA CCC ATG CCA GAA GGA GGA GGG CAG AAT CAT CAC
    A  A  P  M  A  E  G  G  G  Q  N  H  H
    -1  +1                    +5                    +10
171 GAA GTG GTG AAG TTC ATG GAT GTC TAT CAG CGC AGC TAC TGC
13  E  V  V  K  F  M  D  V  Y  Q  R  S  Y  C
           +15                    +20                    +25
    CAT CCA ATC GAG ACC CTG GTG GAC ATC TTC CAG GAG TAC
    H  P  I  E  T  L  V  D  I  F  Q  E  Y
           +30                    +35
252 CCT GAT GAG ATC GAG TAC ATC TTC AAG CCA TCC TGT GTG CCC
40  P  D  E  I  E  Y  I  F  K  P  S  C  V  P
           +40                    +45                    +50
    CTG ATG CGA TGC GGG GGC TGC TGC AAT GAC GAG GGC CTG
    L  M  R  C  G  G  C  C  N  D  E  G  L
           +55                    +60                    +65
333 GAG TGT GTG CCC ACT GAG GAG TCC AAC ATC ACC ATG CAG ATT
67  E  C  V  F  T  E  E  S  N  I  T  M  Q  I
           +70                    +75                    +80
    ATG CGG ATC AAA CCT CAC CAA GGC CAG CAC ATA GGA GAG
    M  R  I  K  P  H  Q  G  Q  H  I  G  E
           +85                    +90
414 ATG AGC TTC CTA CAG CAC AAC AAA TGT GAA TGC AGA CCA AAG
94  M  S  F  L  Q  H  N  K  C  E  C  R  P  K
           +95                    +100                    +105
    AAA GAT AGA GCA AGA CAA GAA AAT ACC TGT GGG CCT TGC
    K  D  R  A  R  Q  E  N  P  C  G  P  C
           +110                    +115                    +120
495 TCA GAG CCG AGA AAG CAT TTG TTT GTA CAA GAT CCG CAG ACG
121 S  E  R  R  K  H  L  F  V  Q  D  P  Q  T
           +125                    +130
    TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC AAG
    C  K  C  S  C  K  N  T  D  S  R  C  K
           +135                    +140                    +145

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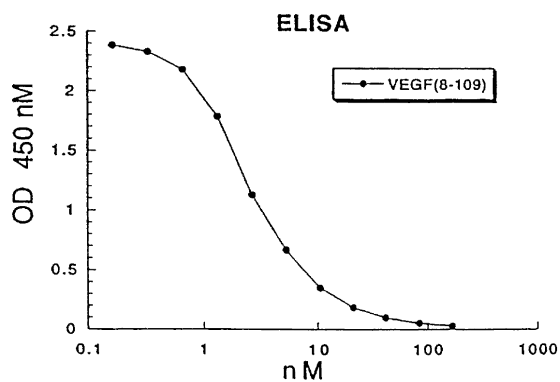
1b

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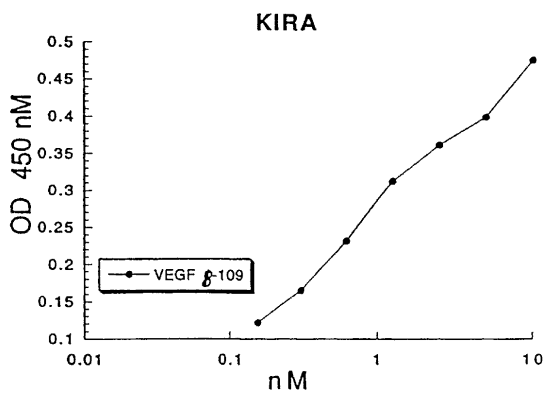
576 GCG AGG CAG CIT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC
148 A  R  Q  L  E  L  N  E  R  T  C  R  C  D
           +150                    +155                    +160
    AAG CCG AGG CCG TGA GCCGGGCA GGAGGAAGGA GCCTCCCTCA
    K  P  R  R  O
           +165
661 GGGTTTCGGG AACCAGATCT CTCACCAGGA AAGACTGATA CAGAACGATC
    GATACAGAAA CCACGCTGCC GCCACCACAC CATCACCATC GACAGAACAG
761 TCCTTAATCC AGAAACCTGA AATGAAGGAA GAGGAGACTC TGCGCAGAGC
    ACTTTGGGTC CCGAGGGCGA GACTCCGGCG GAAGCATTCC CGGGCGGGTG
861 ACCCAGCAGC GTCCCTTTG GAATTGGATT CGCCATTTTA TTTTCTTGC
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961 TGTAGACACA CCGCGGCCGC CAGCACACTG

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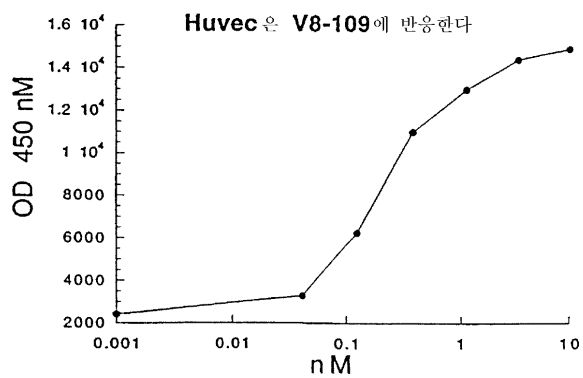
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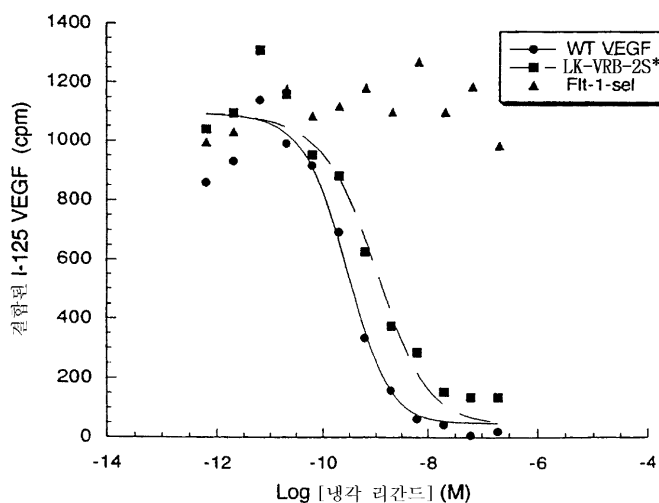
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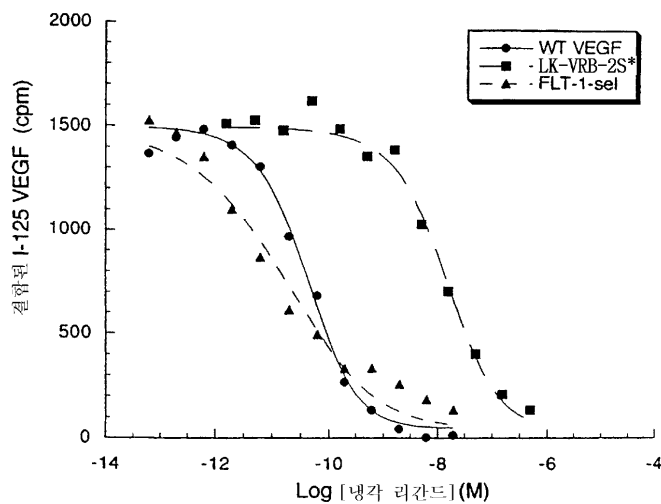
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5



6

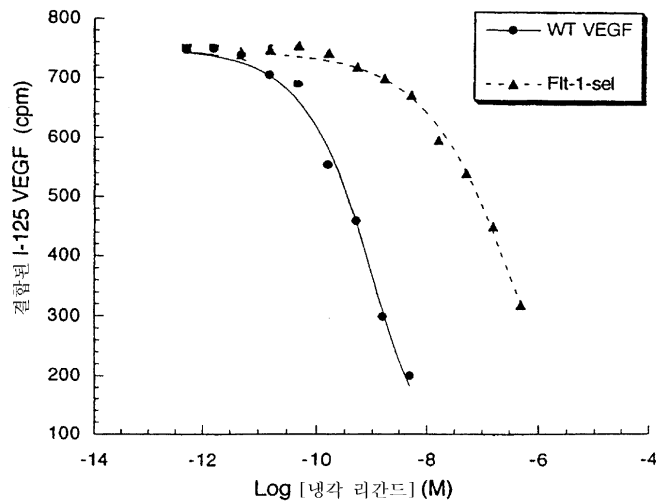


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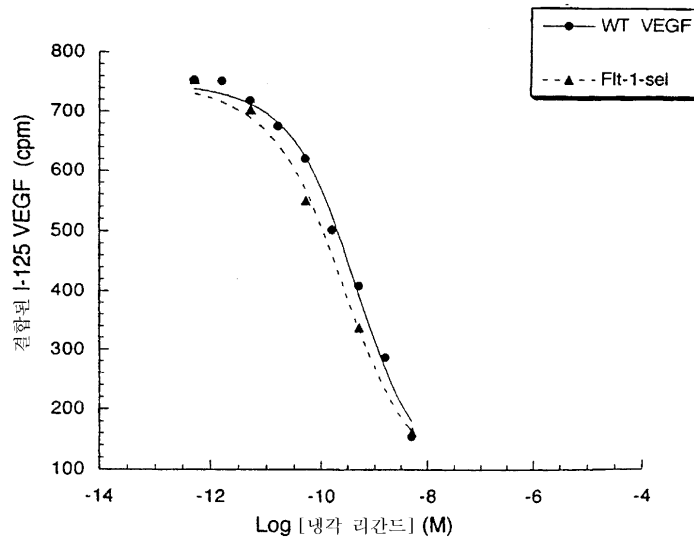
VEGF 알라닌 변이체 결합의 감소율

관기	KDR(1-3)	Flt(1-3)
VEGF(1-109)	1 (10 nM)	1 (6 nM)
20s 나선형:		
Lys 16	1	1
Phe 17*	45	34
Met 18**	5	9
Tyr 21**	19	29
Gln 22**	6	15
Tyr 25**	6	7
40s 루프:		
Ile 43*	21	3
Ile 46*	96	4
Phe 47**	5	3
Lys 48**	1	1
60s 루프:		
Asp 63**	1	8
Glu 64*	10	5
Gly 65**	1	1
Leu 66**	1	10
80s 루프:		
Gln 79*	55	3
Met 81**	9	5
Ile 83*	89	7
His 86**	2	1
Gln 89	1	1
Ile 91	1	1
100s 루프:		
Lys 101	1	1
Glu 103	1	1
Arg 105	1	1
Pro 106	1	1

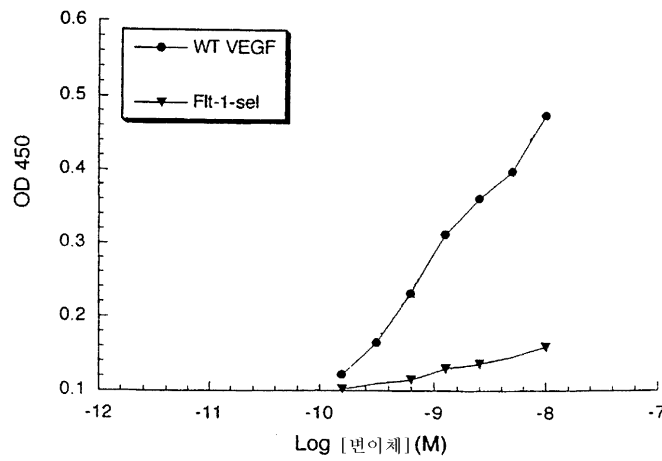
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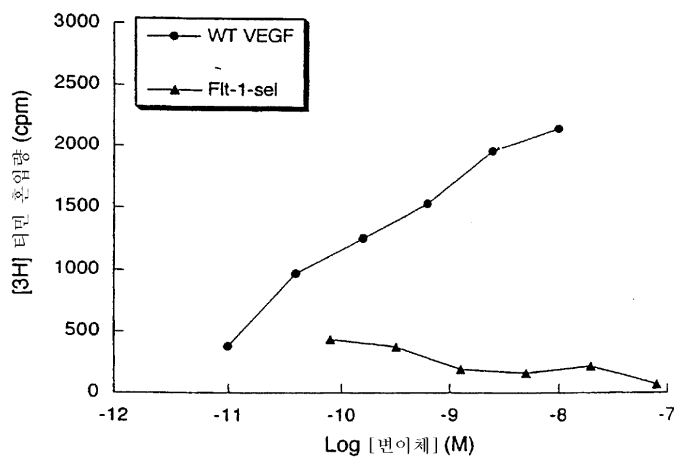
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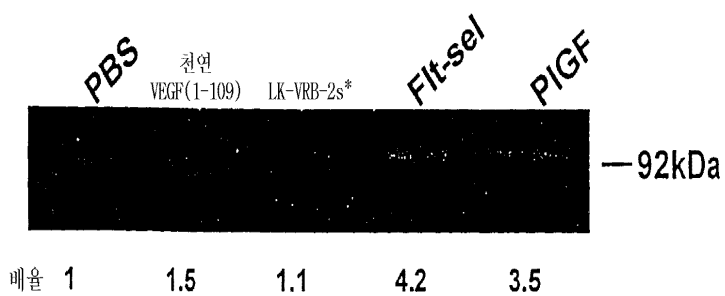
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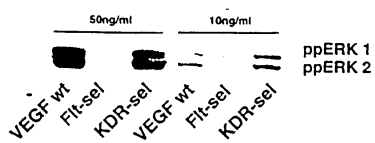
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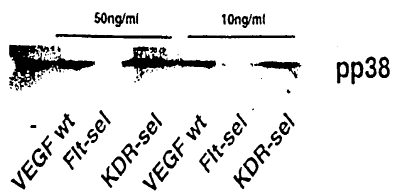
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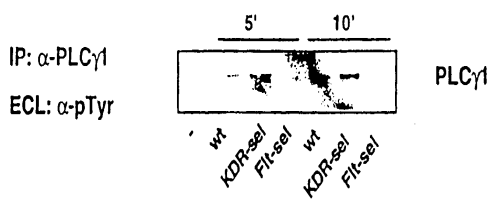
12a



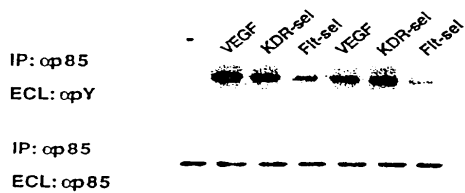
12b



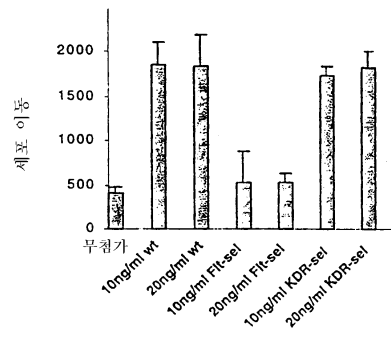
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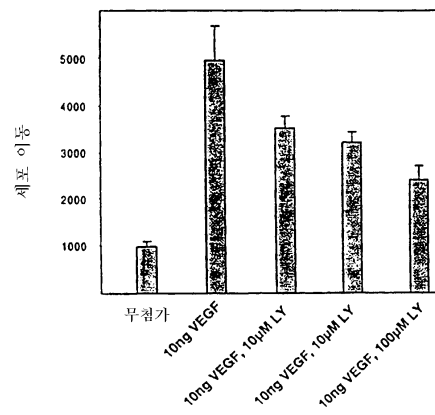
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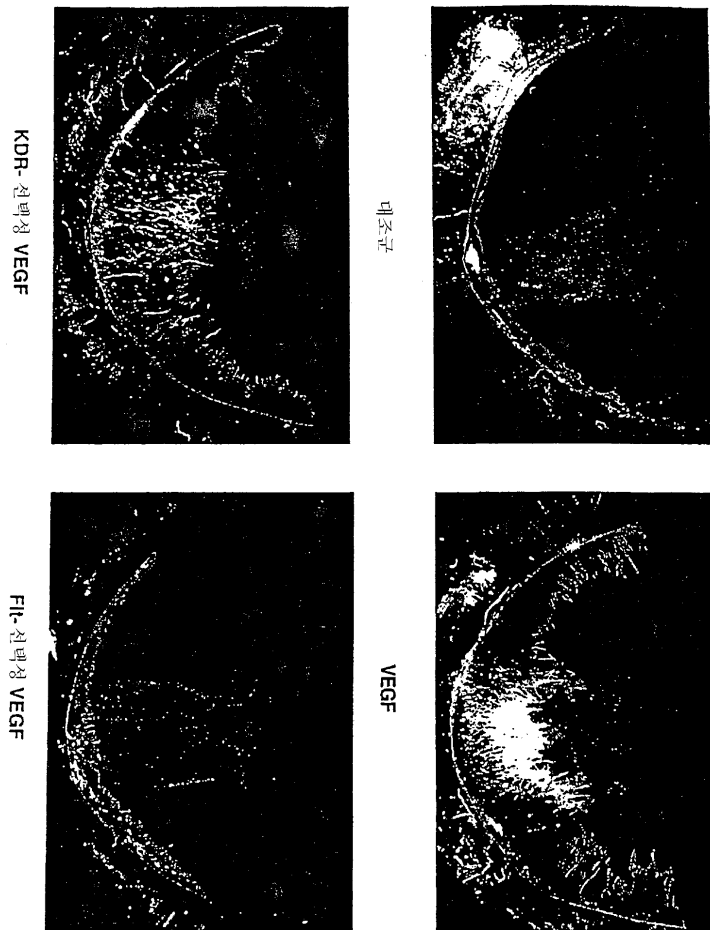
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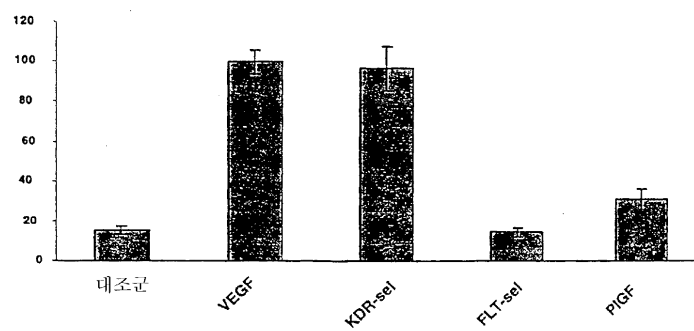
14b



15a



15b



Sequence Listing

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<120> VASCULAR ENDOTHELIAL CELL GROWTH FACTOR VARIANTS AND
USES THEREOF

<130> P1734R1PCT

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<141> 2000-04-10

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 <221> unsure
 <222> 19, 20, 21, 28, 29, 30, 31, 32, 33, 40, 41, 42
 <223> N at indicated positions may be G, A, T or C; S at indicated positions may be C or
 <400> 1
 cacgaagtgg tgaagttcnn sgatgtcnns nnsccgagcn nstgcatcc 50
 aatcgag 57
 <210> 2
 <211> 42
 <212> DNA
 <213> Artificial
 <220>
 <221> Misc_feature
 <222> 1-42
 <223> Sequence is synthesized.
 <220>
 <221> unsure
 <222> 16, 17, 18, 22, 23, 24, 25, 26, 27
 <223> N at indicated positions may be G, A, T or C; S at indicated positions may be C or
 <400> 2
 ggggctgct gcaatnnsa gnnsnnsag tgtgtgccca ct 42
 <210> 3
 <211> 990
 <212> DNA
 <213> Homo sapiens
 <400> 3
 cagtgtgctg gggcccggc gcgagccggc cggcccggg tcgggectcc 50
 gaaaccatga actttctgct gtcttgggtg cattggagcc tcgccttgct 100
 gctctacctc caccatgcca agtgggccca ggctgcaccc atggcagaag 150
 gaggagggca gaatcatcac gaagtgggtg agttcatgga tgtctatcag 200
 cgcagctact gccatccaat cgagaccctg gtggacatct tccaggagta 250
 ccctgatgag atcgagtaca tcttcaagcc atcctgtgtg cccctgatgc 300
 gatgcggggg ctgctgcaat gacgagggcc tggagtggtg gccactgag 350
 gagtccaaca tcaccatgca gattatgcgg atcaaacctc accaaggcca 400
 gcacatagga gagatgagct tcctacagca caacaaatgt gaatgcagac 450
 caaagaaaga tagagcaaga caagaaaatc cctgtggggc ttgctcagag 500
 cggagaaagc atttgtttgt acaagatccg cagacgtgta aatgttcctg 550

