

(19) (12) (KR) (B1)

(51) 。 Int. Cl. ⁶ (45) 2002 06 20
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(21) 10 - 1994 - 0024025 (65) 1995 - 0008536
 (22) 1994 09 23 (43) 1995 04 19

(30) 08/126.974 1993 09 24 (US)

(73)

(72)

-
 10956 20
 10950 71
 07458 24
 10566

(74)

:

(54)

가 , 1 가 . 1
 가 , 1 가 . 1

armacol. 95 (1) : 369 - 418, Springer, 1990). (Baird, A., Bohlen, P., Handbook of Exp. Pha

(, No. 4, 378,347 to Franco).

, FGF - 2 FGF ,
 , FGF -
 , FGF 가 FGF - 2 가 FGF - 2
 (E. coli) (S. cervisiae) , 2 3

(presta) , FGF - 2 - 118,
 - 119, - 128, - 129
 ,
 M6B - bFGF
 (Presta, M., , Biochem. Biophys. Res. Com. 185 : 1098 - 1107 (1992)). 가,
 8 - 129 , 70% FGF - 2 - 12
 M6A - bFGF
 (Eur. Pat. Ap, Pub. No. 363, 675 to Bergonzni, L.)

FGF , FGF - 2 (33 - 77) (102 - 129)가 , FGF
 (Baird, A., . P
 roc. Nat. Acad. Sci. USA 85 : 2324 - 2328 (1988)). , FGF - 2 (115 - 12
 4) . FGF - 2
 (Zhang, J., , Droc. Nat. Acad. Sci. USA 88. 3446 - 3450 (1991) Eriksson, E.
 A., , Proc. Nat. Acas. Sci. USA 88 : 3441 - 3445 (1991)). , - 128 - I
 38, - 129 - 134 (9 - 154
 - 119 - 129, - 120 - 125)
 2 (Zhang,).
 , FGF 가 .

- 2

가
 가 .
 , 가 ;
 가
 가 .
 - 128 - 138, - 2 , - 128, - 138,
 - 129, - 134, - 129 - 114 가
 - 2 - 128 - 138,
 가 .

DNA, DNA
 DNA , 가

1A 1B FGF - 2 (1A) gln^{128, 138} FGF - 2 (1B)
 2 FGF - 2 (BHK) FGF - 125
 I FGF - 2 FGF - 2
 ; gln¹³⁸ FGF - 2 ;
 gln^{128, 138} FGF - 2 ; gln¹²⁸ FGF - 2 .
 3A 3B FGF - 2 . 3A 1 ((- 129 - 134) .
 - 128 - 138) , 3B 2 (.
 4 - FGF - 2 (10ng/ml) .
 5 I FGF 2 (10ng/ml) .

FGF
 (FGF) " " , 가 , " (aglycan)"
 , " (porteoglycan)" , D - D -
 가 , 가 가 . " "
 (mutein) , .

가
I 가 ; 가 . , (BHK)
가 , ,
(BHK)

가 . , , ,

가 .

FGF - 2 . " FGF - 2" 146 -
, 154 , FGF ,
- 2 .
DNA , " " FGF - 2
FGF - 2 .

FGF - 2 . FGF - 2 , FGF - 2 가,
가
N - C - FGF
FGF - 2S , Eur. Pat. Ap. Pub. No. 281,822, Seno 가
가 ; FGF - 2

(Eur. Pat. Ap. Pub. No. 320, 148, Arakawa Fox) ; Eur. Pat, Ap. Pub. No. 326, 907, Seno 7 -
46 가 가
; Eur. Pat. Ap. Pub. No. 298, 723, Fiddes N - I 가
; Eur. Pat. Ap. Pub. No. 363, 675, Bergonzoni
M1 - bFGF - M6 - bFGF : Seddon Glu FGF Ala - 3 S
er - 5 FGF : 9 - 가 10 - 가
08/126,973 2 3 가 ;

FGF - 2

FGF - 1 FGF - 2 , FGF - 1 FGF FGF - 2 FGF

FGF - 2 Zhang 2 가 FGF - 2 (gln¹²⁸ FGF - 2),
 - 128 가 FGF - 2 (gln¹³⁸ FGF - 2),
 - 138 가 FGF - 2 (gln^{128, 138} FGF - 2)

Gln^{128, 138} FGF - 2 FGF - 2 가
 , gln¹²⁸ FGF - 2 gln¹³⁸ FGF - 2 10 가 , gln^{128, 138} FG
 F - 2 100 가 BHK

FGF - 2 가 , gln¹²⁸ - FGF - 2 gln¹²
 8, 138 - FGF - 2 10 , gln¹³⁸ FGF - 2

가, (ABAE) gl
 n^{128, 138} FGF - 2 ABAE , Gln¹²⁸ FGF - 2

1 | 가 FGF - 2 FGF - 2
 - 129 가 FGF - 2 (gln¹²⁹ FGF - 2),
 - 134 가 FGF - 2 (, gln¹³⁴ FGF - 2), - 129
 - 134 가 FGF - 2 (gln^{129, 134} FGF - 2) FGF - 2

FGF DNA , DNA
 FGF

FGF DNA , 가, /

1
 RNA, RNA DNA
 . FGF RNA RNA DNA
 1
 /
 , FGF - 2 Lys - 134 Arg - 129
 . Lys 138
 gln^{129, 134} FG
 FGF - 2
 F - 2 (agonist) (urokinase) - " "
 가 . FGF FGF
 /
 Lys - 138 , gln^{128, 138} FGF - 2 Arg - 128 (plasminogen)
 , FGF - 2
 " (antagonist)" , FGFs , FGF , FGF
 가 , 가
 가
 (agonism)
 1
 FGF - 2
 glu^{3.5} FGF - 2 Seddon A.P., , Annals N.Y. acad. Sci. 638: 98 - 108(199
 1) , pUC 18 FGF - 2 155
 British Bio - technology, Oxford, UK (2 - 49) pUC
 I8 Hind III BspM II FGF - 1 5 N -
 Ndel pUC 18 , 3 5
 FGF - 2 cDNA pUC I8 Ndel BamHI , pET - -
 3a , pET - (M13) PS Ndel BamHI

(Rosenberg, A.H., , Gene 56: 125 - 135 (1987))

-가 DNA 2 , 5' - GTCTAGAAAA TACACCAGTT GGTACGTAGC ACTGCAGCGA ACCGG TCAGT AATAA - 3' (SEQ ID NO 1) 5' - CTTGGTTCCC AACAGGGCC CGGGCAGAAA GCTA - 3' (SEQ ID NO 2) glu¹²⁸ - FGF - 2
SnaBI, Pst 1 Apal () . Wells, (G ene 34: 315 - 323 (1985))가 DNA

5' - GTAGCACTTA AGCGTACGGG GCAGTATAAG CTTGGTTCCC AACAGGGC C - 3' (SEQ ID NO 3) 5' - CTGTTTGGGA ACCAAGCTTA TACTGCCCG TACGCTTAAG TGCTAC - 3' (SEQ ID NO 4), 5' - GTAGCACTGC AGCGTACGGG GCAGTATAAG CTTGGTTCCC AACAGGGCC - 3' (SEQ ID NO 5) 5' - CTGTTTGGGA ACCAAGCTTA TACTGCCCG TACGCTGCAG TGCTAC - 3 (SEQ ID NO 6) 2 SnaBI Apa 1 Afl 2 H ind 3 () gln¹³⁸ FGF - 2, Hind 3 () gln^{128, 138} - FGF - 2

SnaBI Hind 3 5' - GTAGCACTGC AGCAGACGGG GCAGTATC - 3' (SEQ ID NO 7), 5' - AGCTGATACT GCCCGTCTG CTGCAGTGCT AC - 3' (SEQ ID NO 8) glu¹³⁸ FGF - 2 Pst 1 () 4 glu^{128,129,134,138} FGF - 2 SnaBI Apa 1 5' - GTAGCACTTA A GCAGACGGG GCAGTATCAG CTTGGTTCCA AACAGGGCC - 3' (SEQ ID NO 9), 5' - CTGTTTGGGA ACC AAGCTGA TACTGCCCG TCTGCTTAAG TGCTAC - 3' (SEQ ID NO 10) gln¹²⁸ - FGF - 2 - KI38Q Af 2 () gln^{129, 134} 34 FGF - 2 2 Af 2 Apal 5' - TAAGCAGAC GGGGCAGTAT AAGCTTGGTT CCAAACAGG GCC 3' (SEQ ID NO 11) 5' - CTGTTTGGGA ACCAAGCTTA TACTGCCCG TCTGC - 3' (SEQ ID NO 12) Hind 3 () 가 gln¹²⁹ - FGF - 2 , 5' - TTAAGCGTAC GGGGCAGTAT CAGCTTGGTT CCAAACAGG GCC - 3' (SEQ ID NO 13), 5' - CTGTTTGG A ACCAAGCTGAT ACTGCCCGT ACGC - 3' (SEQ ID NO 14) Sp 1 () 가 gln¹³⁴ FGF - 2

7 (E. coli) BL21 Plys S 50 ug/ml 30 ug/ml (Luria broth) 37 , 600 nm 가 0.4가 . 37 2 2 mM 가 . 1 , 0.1 mM EDTA 0.6 M NaCl , 50 mM tris - HCl, pH 7.5 3 0ml , 4 20 (10 ug/ml) (6 x 30) . (10,000 x g, 20) , 1 4 (Pharmacia/LKB) 5ml . 0.8 um (Nalgene) , 0.6 M NaCl , 10 mM Tris - HCl pH 7.4 , 3M NaCl (25 ml) (Tris) . 3M NaCl 6 - TSK 5PW (0.21 x 15 cm; the Nest Group, MA) 3 ml/ 90 (0.6 - 2M NaCl)

(BL 21 Plys S) American Type Culture Collection (A.T.C.C) . gln¹²⁸ - FGF - 2 A.T.C.C. 69419 가 , gln¹³⁸ - FGF - 2 A.T.C.C, 69420 가 , gln^{128, 138} - FGF - 2 A.T.C.C. 69418 가 .

2

I FGF - 2

FGF - 2 123

(Zhang,),

(BCDS) FGF - 2 FGF - 2

가

, 290 nm 350nm

Perkin - Elmer LS - 5

2mM

, 10 mM Tris - HCl, pH 7.4 FGF 2ml

(Hepar Industries, Franklin, OH)

BCDS (American Maize - Products, Hammand, IN)

(2 - 5 μ l) 가

가

BCDS가

가

. FGF - 2 gln^{128, 138} FGF - 2

370 nM 280 nM

1A 1B

가

, Trpl22 ,

FGF (FGF - 1)

BCDS

가 FGF - 2

(BCD) 가

가

(K_D)

1

FGF , C_t FGF

, F₀

, F₁

, F

1:1

, K₀

, L_t

Kaleidagraph

$$(1) \quad F_0 = F_1 + \frac{\Delta F ((C_t + K_0 + L_t) - \sqrt{(C_t + K_0 + L_t)^2 - 4C_t L_t})}{2C_t}$$

가 , 1 1 - 20

FGF 가 FGF

가

, BCDS

14

가

7 -

FGF

가

가

가

가

FGF

$$(2) \quad F_0 = F_1 + \frac{\Delta F ((C_t + K_0 + L_t) - \sqrt{(C_t + K_0 + nL_t)^2 - 4nC_t L_t})}{2C_t}$$

n 가 .

I 2 , K_D n 3
 4 (n=4) FGF - 2
 gln^{128, 138} FGF - 2 4
 5000 , 20 , 4 - 6 가 FGF
 . 2 - 4 FGF BCDS (2226 가)
 가 Mach, (Biochemistry 32 : 5480 - 5489 (1993))
 1:4 - 6

I BCDS FGF
 1 FGF - 2 (K_D)

FGF	K _D 헤파린 (nM)	K _D BCDS (nM)
FGF-1	2 ± 0.2	4 ± 2
FGF-2	14 ± 6	14 ± 7
gln ¹²⁸ FGF-2	134 ± 34	178 ± 44
gln ¹³⁸ FGF-2	126 ± 53	228 ± 23
gln ^{128,138} FGF-2	1280 ± 380	1700 ± 600
gln ¹²⁸ FGF-2	3 ± 3	3 ± 1
gln ¹³⁴	2 ± 0.2	12 ± 0.1
gln ^{128,134} FGF-2	8 ± 6	130 ± 27

2 I K_D
 . gln^{128,138} - FGF - 2 gln¹³⁸ FGF - 2 10
 gln^{128,138} - FGF - 2 100 - . BCDS

gln¹²⁸ FGF - 2 FGF NaCl ,

3

I FGF - 2 .

Moscattelli (J. Cell Physiol. 131 : 123 - 130 (1987)) , FGF
 , (BHK) ¹²⁵I - FGF - 2(Amersham Corp.)
 , 24 - 1 FGF - 2
 50 pM¹²⁵ I - FGF - 2 39 4
 , - 2 2M NaCl , 20 mM Hepes, pH 7.5
 n X - 100 ¹²⁵ I - FGF - 2 . 0.1 M , pH 8 0.5% Trito
 2 FGF - 2 가

FGF - 2 Gospardarowicz, (Proc. Nat. Acad. Sci. 81 : 6963 - 6967 (1984))

(100 /ml), (100ug/ml) L - (2 mM) 10% (Hyclone, L
 ogan, UT) Dulbecco 's modified Eagle 's medium (DMEM) 0.5 ml 24 - 8000
 가 5 , 2 , DMEM FGF - 2 20 μl
 Coulter counter
 3

ABAE FGF - 2 가 FGF
 I gln¹²⁸ FGF - 2 gln^{128,138} FGF - 2 EC₅₀ (3A) 0.5 ng/ml - 5.6 ng/ml ,
 10 가 , gln¹³⁸ FGF - 2 - 2
 (3B).

FGF - 가 (ABAE)
 96 - 20,000 / FGF - 2 (100
 /ml), (100 ug/ml) L - (2mM) 10% (Hyclone, Logan, UT)
 DMEM .24 , - 0.05% Triton X - 100
 , 60 mM Tris - HCl, pH 8.5 - - (u
 PA) D - - p - (Ame
 rican Diagnostics, Greencoich, CT) , Presta,
 - 4
 gln^{128,138} - FGF - 2 uPA

가 3 - ABAE -
 n Labwares, Bedford, MA) 0.7 mg/ml 1 × DMEM - I (Becton Dickinso
 3 - (24 -) 0.5 ml 가 NaHCO₃ pH
 0 / , (1 - 2 mm) , ABAE 50,00
 (100 /ml), (100 ug/ml) L - (2 mM) 10% 5 ,
 n, UT) DMEM 37 , FGF - 2 (Hyclone, Loga
 37 48 , 가 (- 20)

ABAE , Hamanatsu C2400 Zeiss Axioshop
 가 Kontron IBAS Image Analyzer

3 - 4
 5 gln^{128,138} FGF - 2
 FGF - 2

(1)

(i) : Lu Yuan Li

Andrew P. Seddon

Yakov Gluzman

Peter Bohlen

(ii) :

(iii) : 14

(iv) :

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Patent Law Department

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Wayne, NJ 07470 - 8426

(v)

(A) : 5.25" 360 Kb

(B) : IBM PC

(C) : MS DOS

(D) :

(viii)

(A) : Estelle J, Tsevdos

(B) : 31145

(C) : 854 - 011

(ix)

(A) : 201 - 831 - 3242

(B) : 201 - 831 - 3305

(2) XEQ ID NO : I

(i)

(A) : 55

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) :

(xi) : SEQ ID NO 1:

GTCTAGAAAA TACACCAGTT GGTACGTAGC ACTGCAGCGA ACCGGTCAGT ATAAA

(3) SEQ ID NO: 2

(i)

(A) : 34

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) :

(xi) : SEQ ID NO 2:

CTTGGTTCCC AAAGAGGGCC CGGGCAGAAA GCTA

(4) SEQ ID NO : 3

(i)

(A) : 50

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) SEQ ID NO 4

(xi) : SEQ ID NO 3:

GTAGCACTTA AGCGTACGGG GCAGTATAAG CTTGGTTCCC AAACAGGGCC

(5) SEQ ID NO : 4

(i)

(A) : 46

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 3

(xi) : SEQ ID NO 4:

CTGTTTGGGA ACCAAGCTTA TACTGCCCCG TACGCTTAAG TGCTAC

(6) SEQ ID NO : 5

(i)

(A) : 50

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 6

(xi) : SEQ ID NO 5:

GTAGCACTGC AGCGTACGGG GCAGTATAAG CTTGGTTCCC AAACAGGGCC

(7) SEQ ID NO : 6

(i)

(A) : 46

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 5

(xi) /,l : SES ID NO 6:

CTGTTTGGGA ACCAAGCTTA TACTGCCCCG TACGCTGCAG TGCTAC

(8) SEQ ID NO : 7

(i)

(A) : 28

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 8

(xi) : SEQ ID NO 7:

GTAGCACTGC AGCAGACGGG GCAGTATC

(9) SEQ ID NO : 8

(i)

(A) : 32

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 7

(xi) : SEQ ID NO 8:

AGCTGATACT GCCCCGTCTG CTGCAGTGCT AC

(10) SEQ ID NO 9

(i)

(A) : 50

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 10

(xi) : SEQ ID NO 9:

GTAGCACTTA AGCAGACGGG GCAGTATCAG CTTGGTTCCA AAACAGGGCC

(11) SEQ ID NO : 10

(i)

(A) : 46

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 9

(xi) : SEQ ID NO 10:

CTGTTTTGGA ACCAAGCTGA TACTGCCCG TCTGCTTAAG TGCTAC

(12) SEQ ID NO : 11

(i)

(A) : 43

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 12

(xi) : SEQ ID NO 11:

TTAAGCAGAC GGGGCAGTAT AAGCTTGTT CCAAAACAGG GCC

(13) SEQ ID NO : 12

(i)

(A) : 35

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) : SEQ ID NO 11

(xi) : SEQ ID NO 12:

CTGTTTTGGA ACCAAGCTTA TACTGCCCG TCTGC

(14) SEQ ID NO : 13

(i)

(A) : 43

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

(ix)

(D) :

(xi) : SEQ ID NO 13:

TTAAGCATAC GGGGCAGTAT CAGCTTGGTT CCAAAACAGG GCC

(15) SEQ ID NO : 14

(i)

(A) : 34

(B) :

(C) 가 :

(D) :

(ii)

(A) :

(v) : DNA

4.

Gln^{129,134} FGF - 2

11

FGF -

2

5.

Gln^{128,138} FGF - 2

12

FGF - 2

6.

ATTC 69420

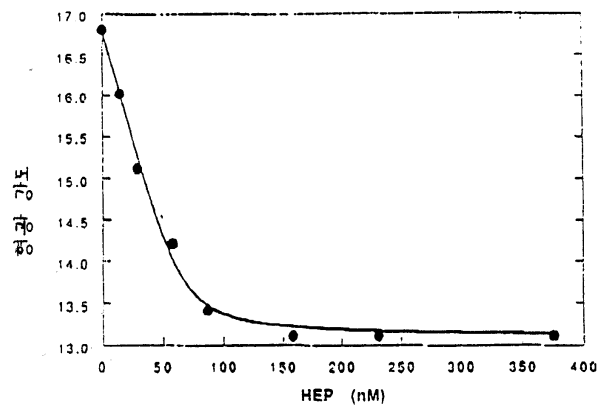
Gln¹³⁸ FGF - 2

7.

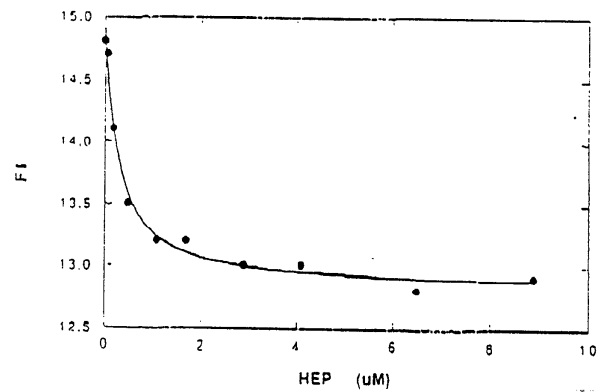
ATTC 69418

Gln^{128,138} FGF - 2

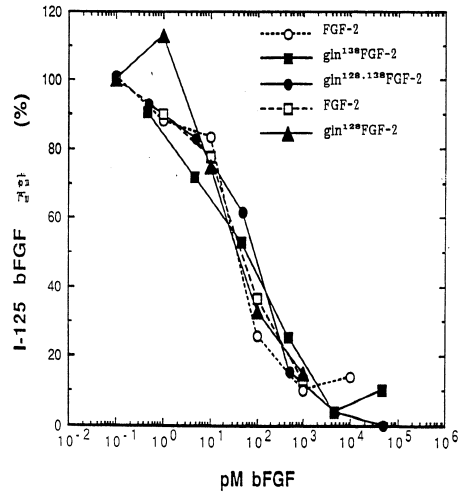
1a



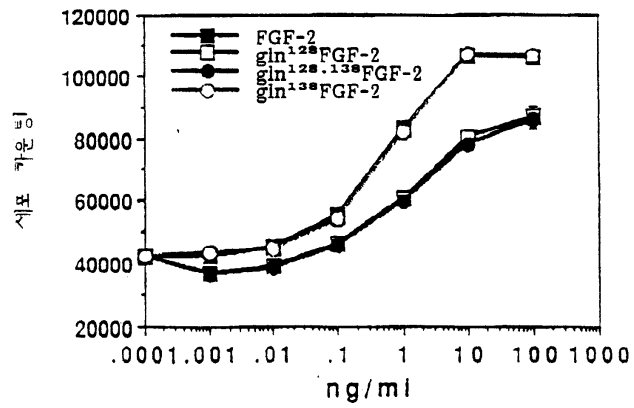
1b



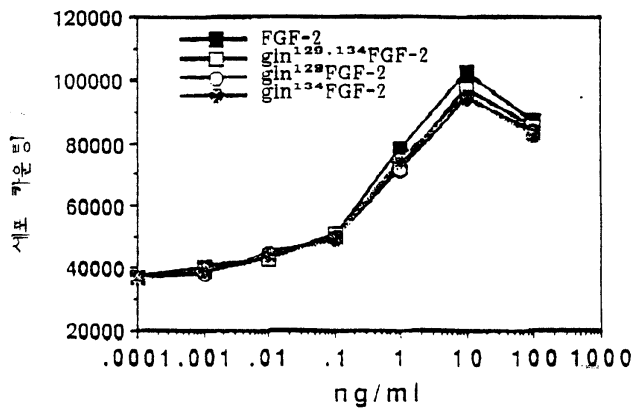
2



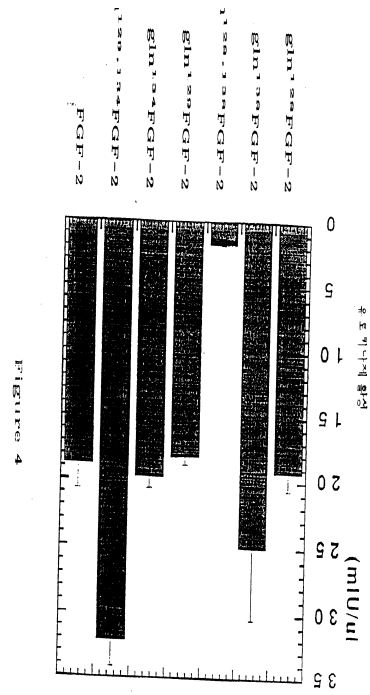
3a



3b



4



5

