

:

(54) - I F N -

PEG IFN - Cys17 PEG - IFN - PEG
PEG , , 가 ,
IFN - PEG

1

35 U.S.C. § 119(e) U.S 60/083,339

- IFN - Cys¹⁷
가 , ,
2 PEG

(IFN -)
-가 RNA 20,000 Da DNA
(Derynk et al., Nature, 285:542 - 547, 1980)
166

Shepard (Nature, 294:563 - 565, 1981) - 842 (141 Cys Tyr)
1119 - 1121

Mark 469 (T) (A) , 17 Cys Ser
(Proc. Natl. Acad. Sci. U.S.A., 81(18):5662 - 5666, 1984). IFN - ' IFN -
(- 70)

(PEO) (PEG)

PEG 가 :
HO - CH₂ - CH₂O(CH₂CH₂O)_nCH₂CH₂ - OH

HO - PEG - OH , - PEG - 가

:

" - PEG - " " - - CH₂CH₂O(CH₂CH₂O)_nCH₂CH₂ - - " . PEG - PEG - OH(m - PEG)

CH₃O - (CH₂CH₂O)_n - CH₂CH₂ - OH

PEG가 . PEG R(-PEG-OH)m , R (m) (branching arm)

PCT WO 96/21469 PE G (CH₃O - PEG -)_pR - X , P 2 3 , R " PEF" PEG PEG

PEG , 가 , Harris U.S. 0 6/026,716 , 가 PEG 가 :

- PEG - CO₂ - PEG - + H₂O - PEG - CO₂H + HO - PEG -

PEG 가 . PEG , PEG : HO - CH₂CHRO(CH₂CHRO)_nCH₂CHR - OH, , R H CH₃ .

PEG . PEG (PEG) , PEG , - , -

PEG - (ADAGEN²) (SCIDS) , PEG L - (ONCAPSP AR²) (ALL) , PEG - (INTRON(R) A) C

PEG - 94 N.L. Burnham, Am. J. Hosp. Pharm., 15:210 - 218, 19

PEG . PEG N - PEG , PEG ,

(zero) , PEG (" P EGmers") , PEG

PEG , 가
 , Katre U.S. 4,766,106 U.S. 4,91
 7,888 , N -
 IFN - IL - 2 PEG
 가 IFN -
 , Cys¹⁷ IFN - Betaseron ?
 PEG 가 (glycosylation)가 가

PEG - IFN - EP 593 868 PEG - IFN -
 , PEG , PEG - IFN -
 가 (Monkarsh et al., ACS Symp. Ser., 680:207 - 216, 1997).

Kinstler EP 675 201 , mPEG - (M
 GDF) N - , 가 PEG 가
 . Gilbert U.S. 5,711,944 , IFN - PEG 가 가
 가

PEG PEG , ,

- PEG 가
 Woghiren PEG - PEG (Bioconjugate Chem., 4(5):3
 14 - 318, 1993). PEG - PEG ()
 PEG

- IFN - , PEG - IFN - Cys¹⁷
 vivo IFN - Cys¹⁷ in
), IFN - pH ()
 가 가
 PEG

1 PEG - IFN - (CE)
 2A - 2C (Superose 12) PEG - IFN - : 2A -
 ; 2B - ; 2C - 3

75kg , 10µg 1mg , 20µg 200µg .

가 가

1: PEG - IFN -

mP EG5k5k - OPSS IFN -

PEG - IFN - , 50mM (pH 3.6) 0.37mg/Mℓ
 IFN - 1.0Mℓ 6M 0.37mg/Mℓ (0.75mg, 3.7×10^{-8} moles) 2Mℓ IFN -
 가 . mPEG_{5K} - OPSS 50 1 IFN - 가 , 37 2 50 1
 (CE) , pEG
 PEG - IFN - (1). 50% PEG - IFN -
 0.22mm (Superose
 12 Superdes 75, Pharmacia) , 50mM , 150mM NaCl, pH 7.0 . 2
 A Superose 12 , PEG - IFN -
 , SDS - PAGE (3). PEG - IFN -
 , " " IFN - PEG - IFN - 가
 (2B). (3) (2C). 4 5 , PEG - IFN -
 MALDI MS

mPE G30K30K - OPSS IFN -

IFN - 50mM (pH 3.6) 0.36mg/Mℓ
 3Mℓ H₂O 36mg mPEG_{30K} - OPSS 3Mℓ IFN - 0.36mg/Mℓ (1.08×10^{-8} m
 oles) 가 , 50 2
 < 30% (Superose 12,
 Pharmacia) , 50mM , 150mM NaCl, pH 7.0 , SD
 S - PAGE

2: PEG - IFN -

IFN - - PEG 가 WISH
 IFN - (PEG) PEG - IFN - . WISH - V
 SV , IFN - - Novick et al., (J. Immunol., 129:22
 44 - 2247(1982)) - WISH . WISH

:

WISH (ATCC CCL 25)

(ATCC V - 520 - 001 - 522), - 70

IFN - γ , InterPharm Laboratories LTD (32, 075 - , Batch # 205035), 82 x 10⁶ IU/Ml,
: 222 x 10⁶ IU/mg

1, PBS(pH 7.4) PEG - IFN -

WISH (Earls MEM + 10% FBS + 1.0% L - + /
(100U/Ml, 100 μ g/Ml))

WISH (Earls MEM + 5% FBS + 1.0% L - + /
(100U/Ml, 100 μ g/Ml))

PBS 5mg/Ml MTT, - 70 .

WISH :

IFN - WISH 2X .

- 96 - WISH IFN - 3 , 50 μ l IFN -
(50 μ l WISH).

WISH /EDTA , WISH , 0.8 x 10⁶
Ml가 .

50 μ l WISH (4 x 10⁴ /) 가 . IFN - 1X .

5% CO₂ 가 24 , 50 μ l 1:10 (WISH) VSV (100% WI
SH 48) - ()
가 .

가 48 , 25 μ l MTT 가 , , 가 2

, 200 μ l 100% 가 .

1 , Soft max Pro Spectromax (Molecular devices) ,
595nm .

[1]

PEG화되고, Mock-PEG화된 IFN-베타 샘플의 항바이러스 활성

IFN-베타 샘플 *	EC50**
PEG-IFN-B 공액체	3.9+/- 0.7 pg/ml
IFN-B	16.4 +/- 1.0 pg/ml

* 아미노산 분석으로 결정한 샘플상의 IFN-B의 원액농도

** EC50(+/- S.D.)은 소프트웨어 프로그램 microcal origin 4.1로 측정하였다.

6 1 , PEG - IFN - IFN - W
 . PEG - IFN - 가 IFN - 4 , W
 ISH 가 , " " IFN - PEG - IFN -
 3: PEG - IFN in vitro
 PEG[30kD] - IFN - PEG[2 x 20 kD] - IFN - 2
 WISH (2). 3 3

[2]

PEG-IFN-B의 상대적인 항바이러스 활성

샘플	상대적인 인터페론 활성*(3번의 연구)			
	분석 1	분석 2	분석 3	분석(S.D.)
PEG[30kD]-IFN-B	3.2 X 이상	3.1 X 이상	1.8 X 이상	3.0 X(0.78)이상
PEG[20 x 20 kD]-IFN-B	4.2 X 이상	1.3 X 이상	0.85 X 이상	2.0 X(1.8)이상

* 각 분석에 포함된 표준 IFN-B와 비교한 EC50 투여량

** 330ug/ml의 IFN-B 농도에 기초한 비교. PEG[30kD]-IFN-B(5.41 ug/ml)와 PEG[2 X 20 kD]-IFN-B(6.86 ug/ml)의 원농도는 AAA로 측정하였다.

PEG - IFN - ¹²⁵I - IFN - 2a 가 . IFN - 2a
 T ¹²⁵I Sephadex G25
 , ¹²⁵I IFN 2a (Pharmacia). ¹²⁵I - IFN - 2a IFN - 2
 a ELISA (Biosurce, USA) , Daudi
 , 2 x 10⁶ PEG - IFN - IFN - 2a 3
 0.5 nM ¹²⁵I - IFN - 2a , 2% 0.1%
 RPMI 1640 ,
 IFN - , PEG[30kD] - IFN - PEG[25 x 20kD] - IFN - 7

, Daudi (B) - (3). IFN 200ng/Ml
 2x 가 , CO₂가 100μl 3 . 1 x 10⁵ / (100μls)
 / 가 . 72 37 72 . 48 , (³H) 20μl 1 μCi
 PEG 가 IFN Tomtek . 3 IFN -
 IFN - RP - HPLC) (PEG - IFN

[3]

Daudi 항-증식 분석

	IC50 투여량*	접합 증가 vs IFN
IFN-B(평판 1)	1153.1	--
PEG[30kD]-IFN(71A)	695.6	1.6X
IFN-B(평판 2)	1005.8	--
PEG[40kD]-IFN(71B)	629.4	1.7X

* pg/ml

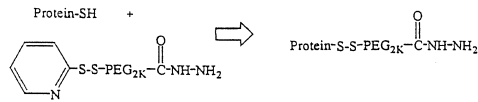
4:

100ng IFN - , PEG[30kD] - IFN - PEG[2 X 20kD] - IFN -
 . IFN - IFN - ELIAS(Toray Industries) , 8
 28 B6D2F1 (6-8)(20g) 4 : 1 9
 500ng/Ml IFN - 200μl (100ng) ; 2 9
 200μl PEG30kD - IFN - ; 3 200μl PEG(2 x 20kD) - IFN -
 ; 4 3 (200μl/) ,
 - 1
 - 70 . Total
 IFN - (AUC) PEG - IFN IFN -
 PEG - IFN IFN - , PEG[2 x 20 kD] - IFN - PEG[30kD] - IFN -

IFN - PEG - IFN(100ng/) . 9 (AUC) IFN -
 , PEG - IFN
 AUC PEG - IFN

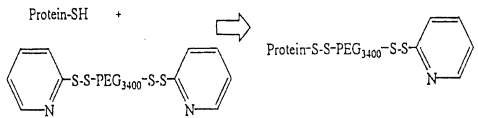
5: PEG

OPSS - PEG_{2K} -



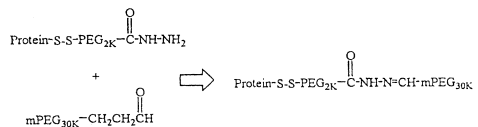
3M ℓ IFN - 0.33mg/M ℓ (0.99mg) 가 , 45 1
 - 50mM (pH 3.8) 0.33mg/M ℓ . 2M ℓ
 3.6mg (40) PEG (OPSS - PEG 2K -)
 90 - 97% ,
 150mM NaCl, pH 7.0 , SDS - PAGE , 5mM ,
 PEG

(OPS S)22 - PEG34003400



6: PEG 2 PEG
 - 50mM (pH 3.8) 0.33mg/M ℓ . 2M ℓ
 6.1mg (40) PEG ((OPSS) 2 - PEG3400) 3M ℓ
 - 0.33mg/M ℓ (0.99mg) 가 , 50 2
 - SDS - PAGE ,
 > 95% , (Superdex 75, Pharm
 acia) , 50mM , 150mM NaCl, pH 7.0 , SDS - PAGE
 PEG

mPE G30k30k - (ALD) IFN - S - S - PEG2k2k -



5 IFN - S - S - PEG 2k - 20 mPEG30k - ALD 가
 4 (25) , (Superose 6, Pharmacia) 가 ,
 PEG > 80%

가 가 , 가 가

가

가

가

가

(57)

1.

- Cys¹⁷

2.

1 ,

3.

2 , (PEG)

4.

1 3 , - - -

5.

1 - - ,

Cys¹⁷

6.

5 , - - PEG

7.

5 6 , - - .
8.

5 6 , - .
9.

5 6 , - , , , , .
10.

5 6 , - - .
11.

5 , - 가 pH .
12.

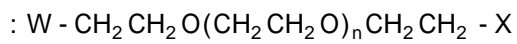
1 3 - - 가 , .

13.

, , 가 , , 12 .

14.

(PEG)



PEG

, , W X , , ;

PEG

EG

PEG

PEG

, PEG -

PEG

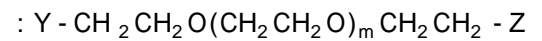
P

15.

14 , , Y , X

PEG

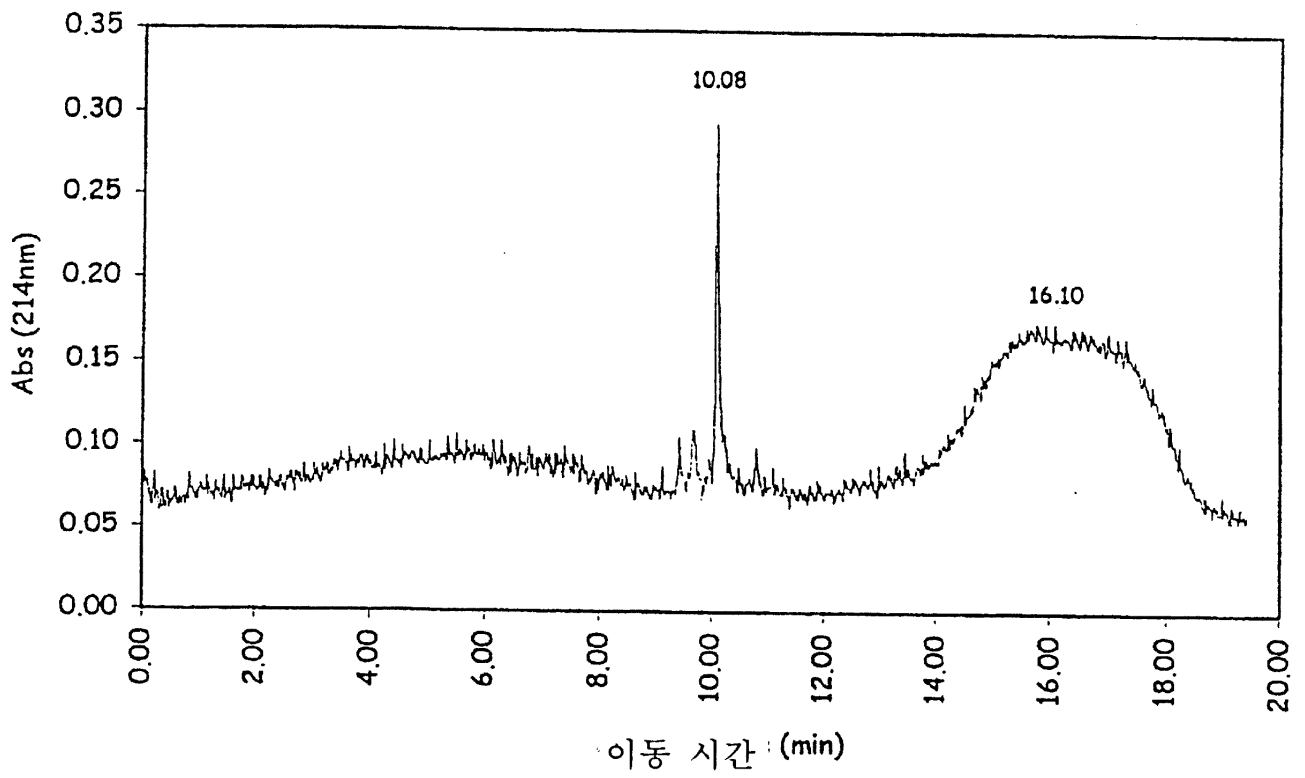
PEG



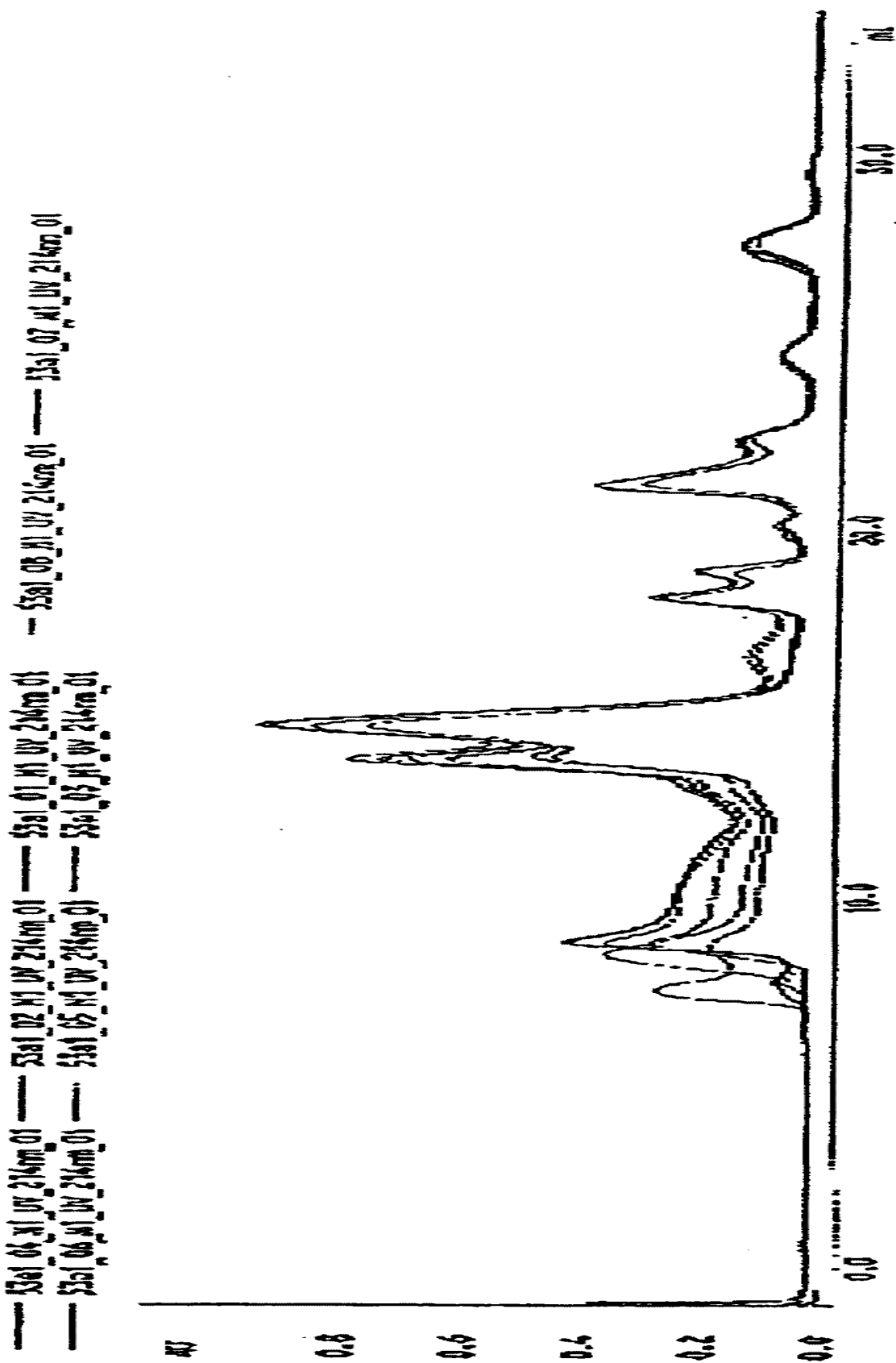
, Z - OCH₃

16.

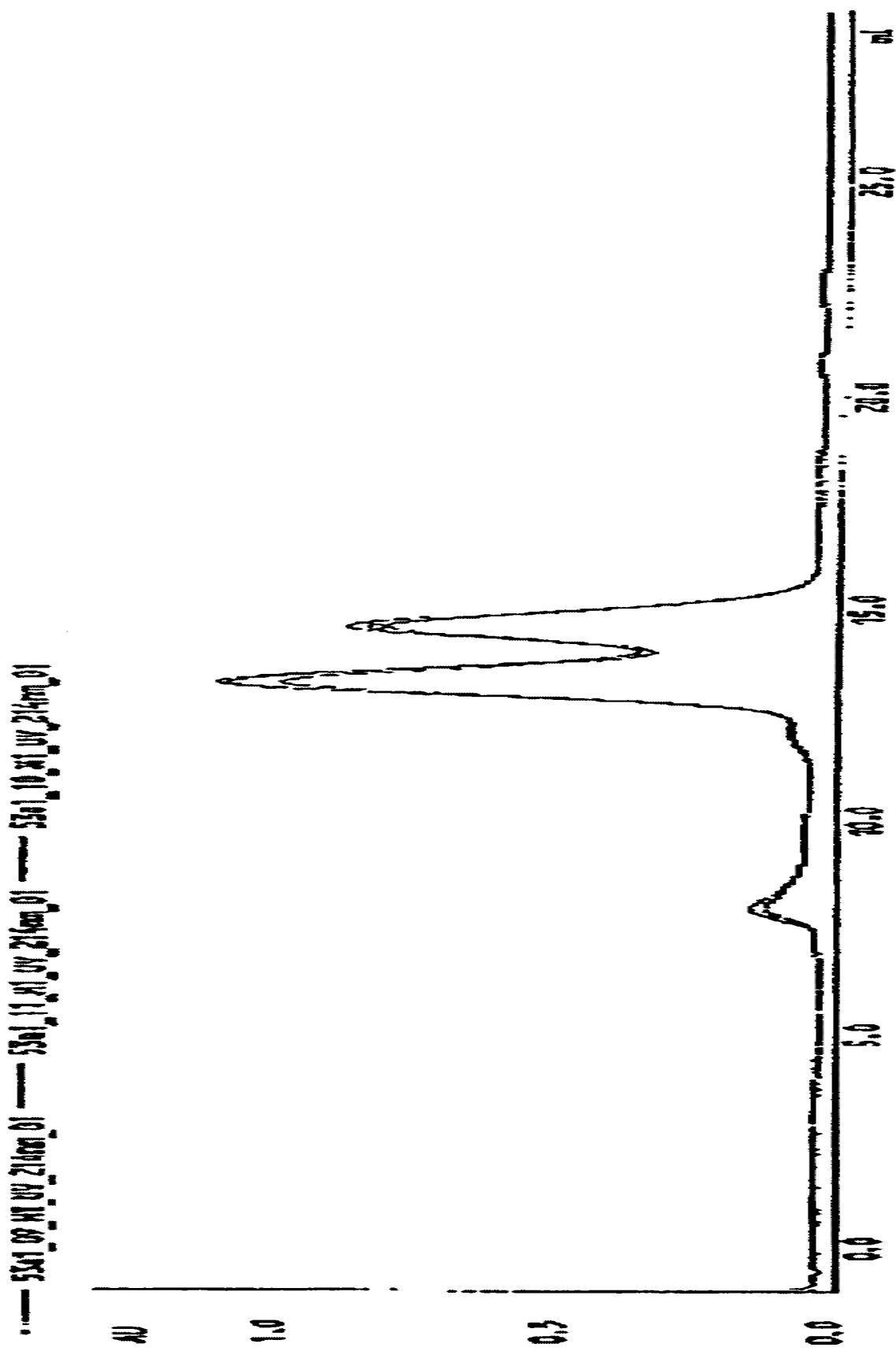
1



2a

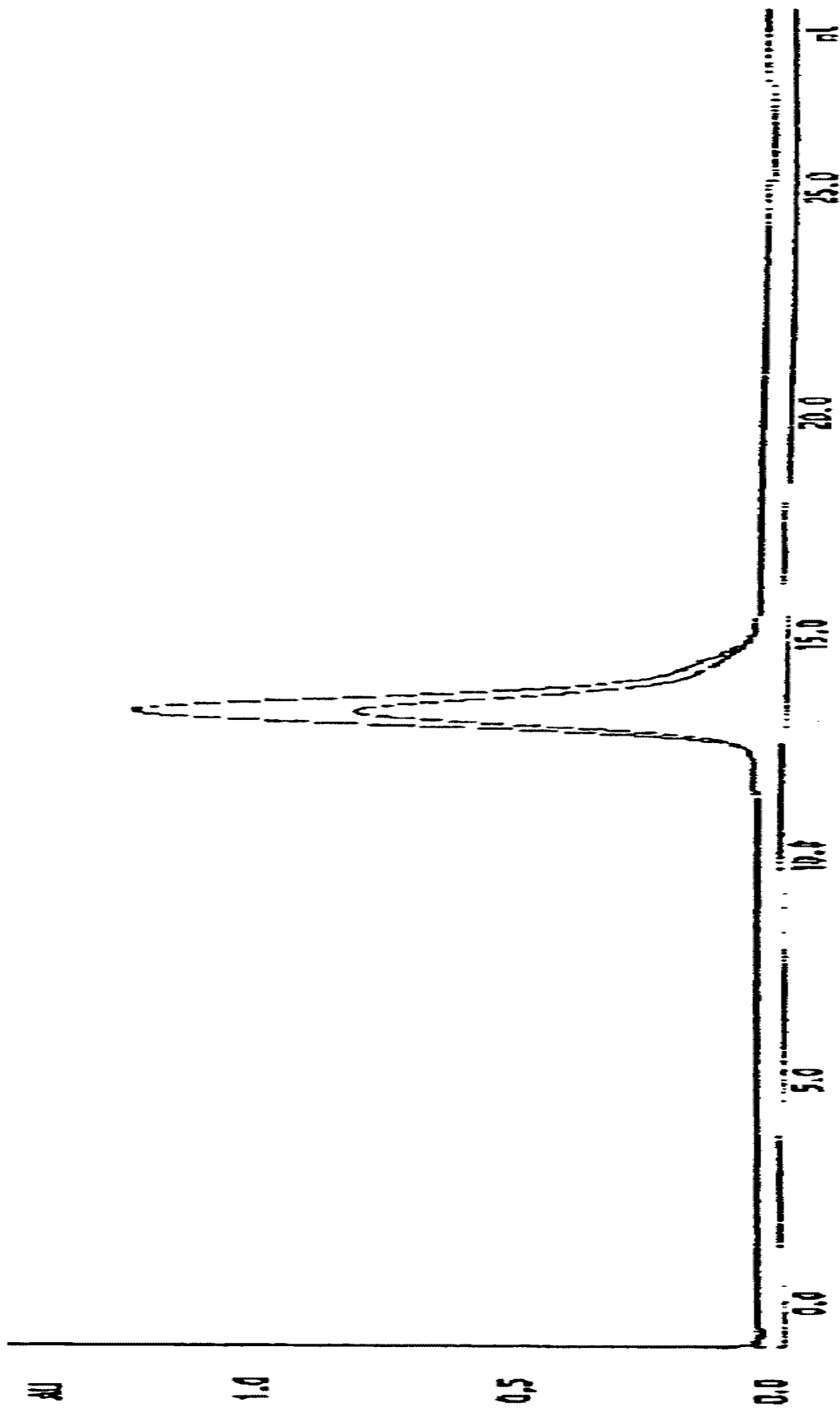


2b

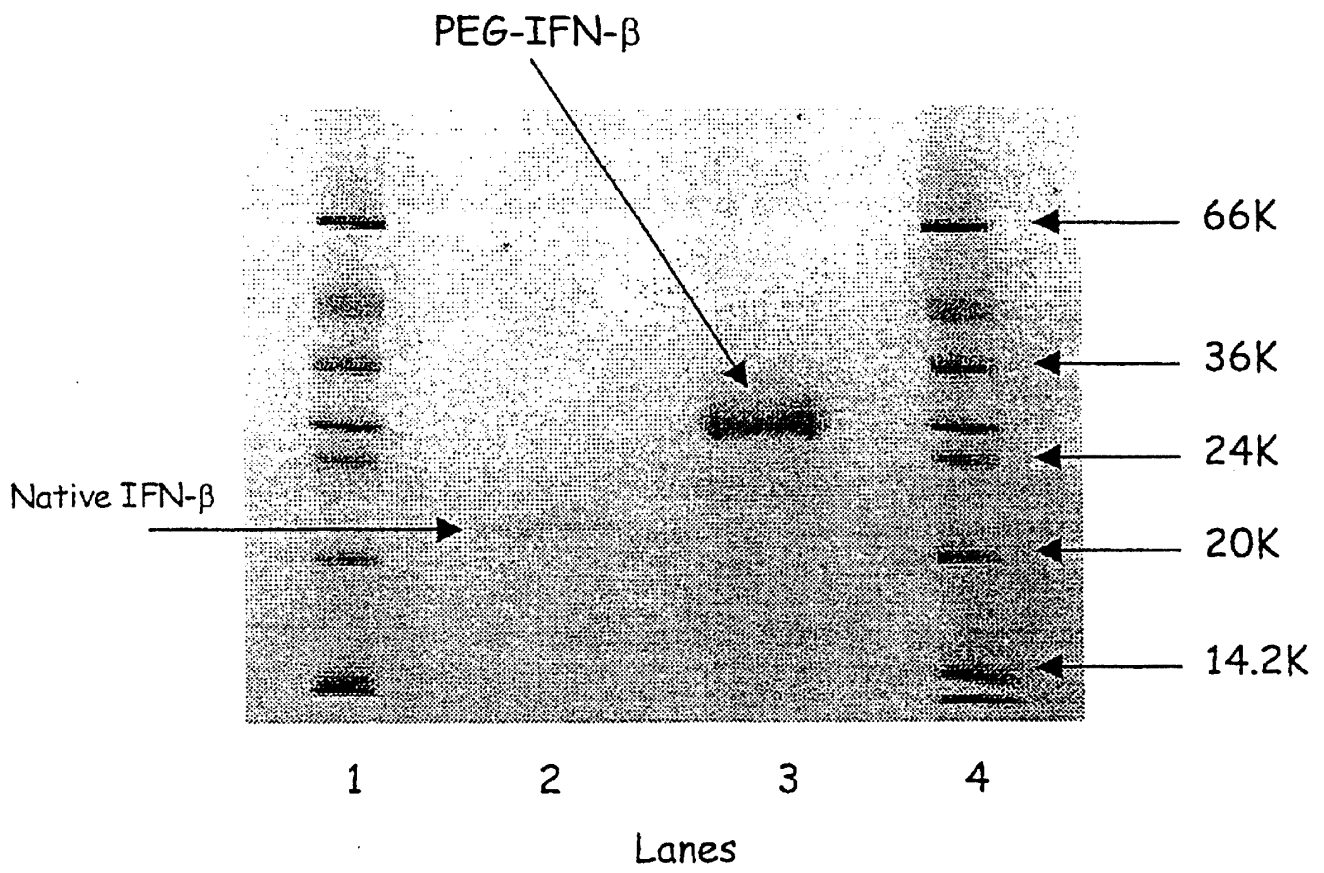


2c

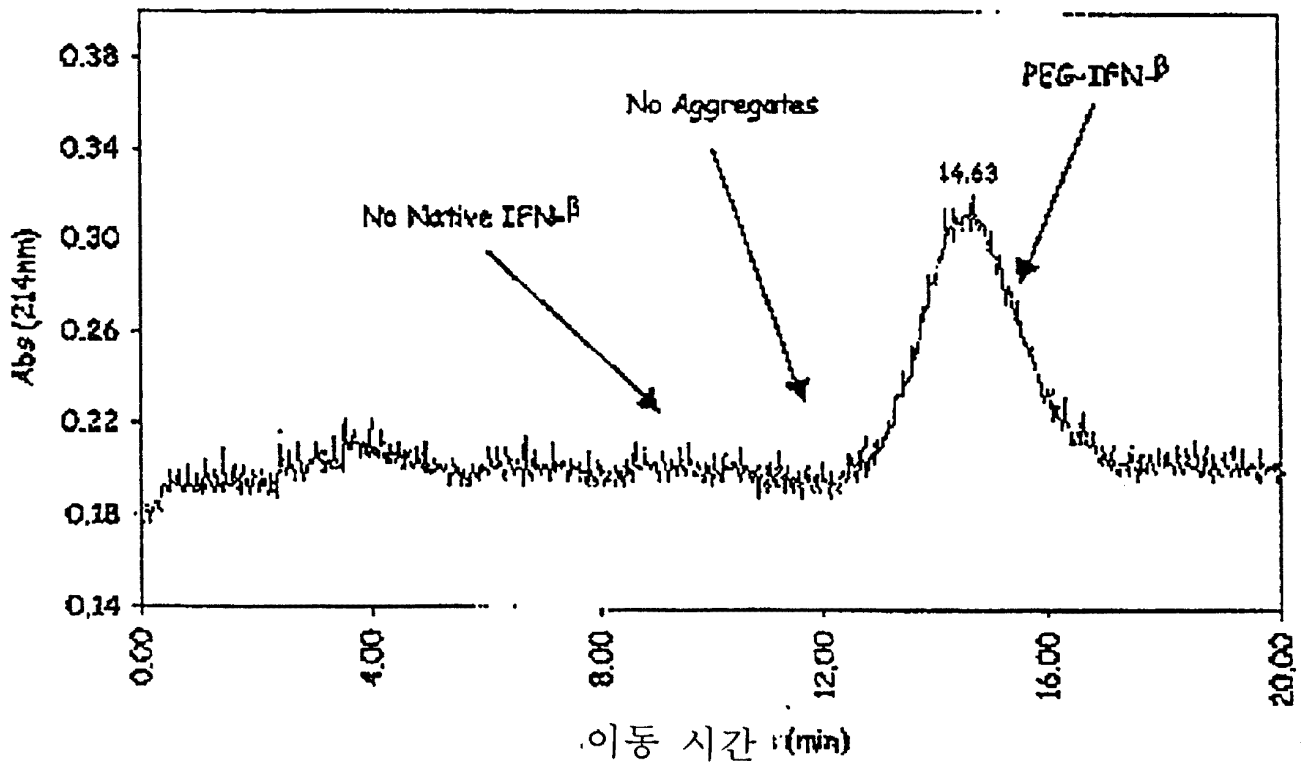
.. 5/21/12 11:07:21 AM 01 5/21/12 11:07:21 AM 01



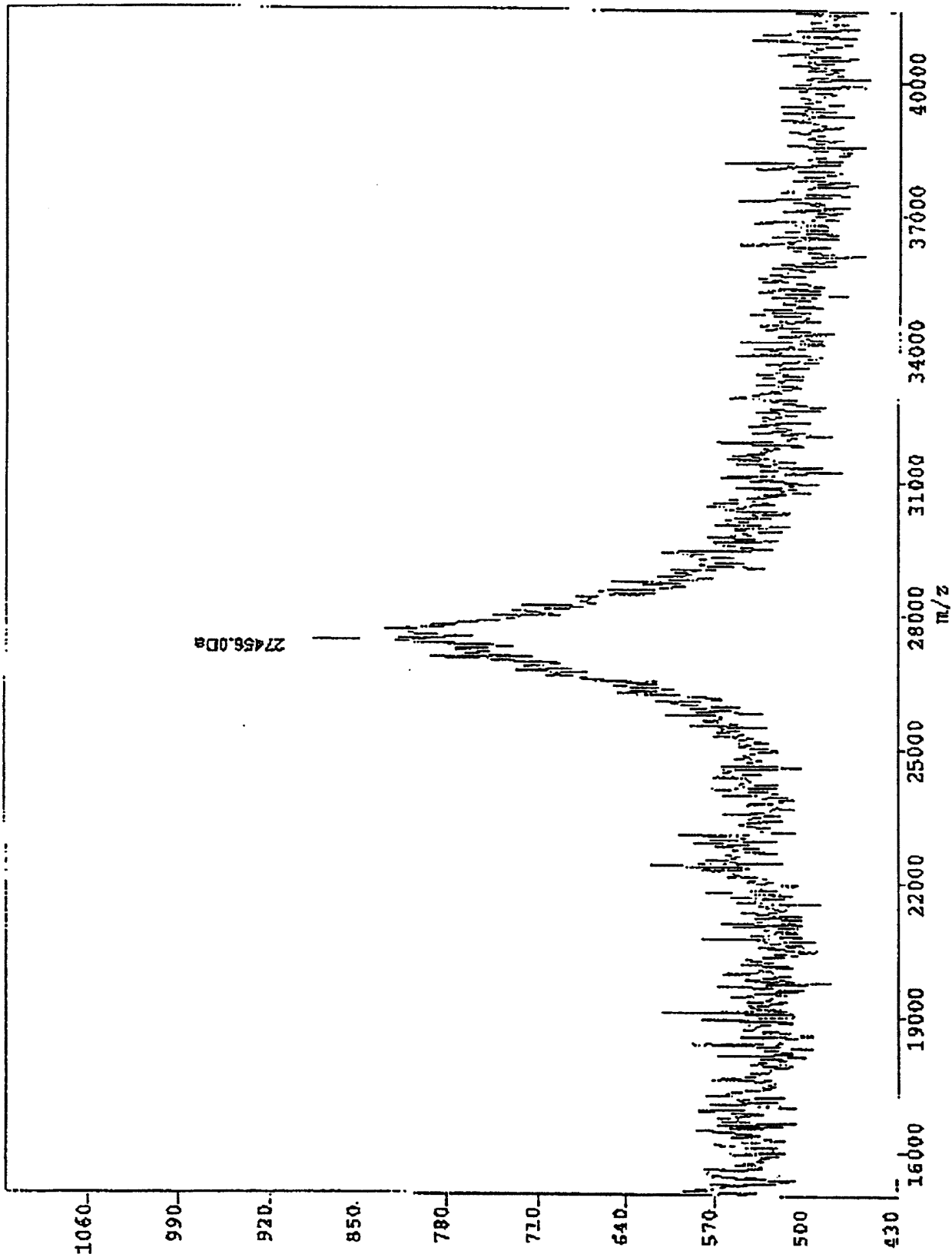
3



4



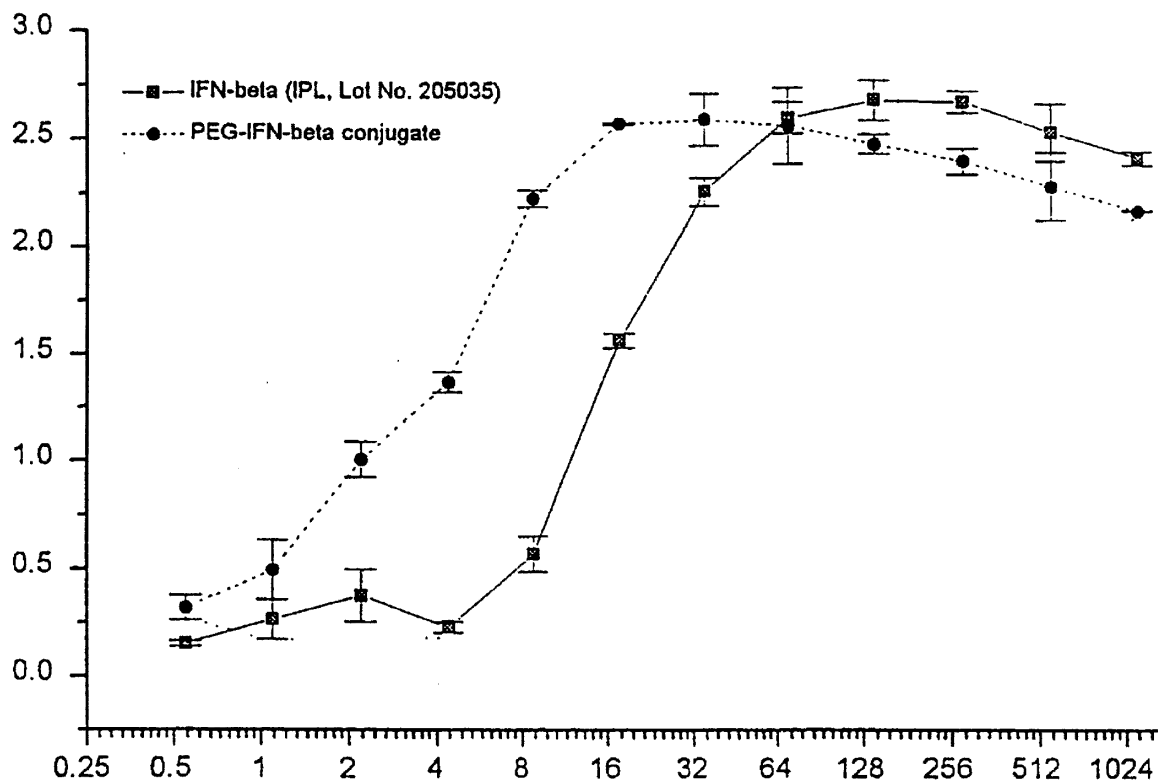
Laser Energy 5.95 (0.06) uJ Vacuum 1.06e-006 torr Polarity Positive Matrix Sinapinic Acid



A b u n d a n c e

6

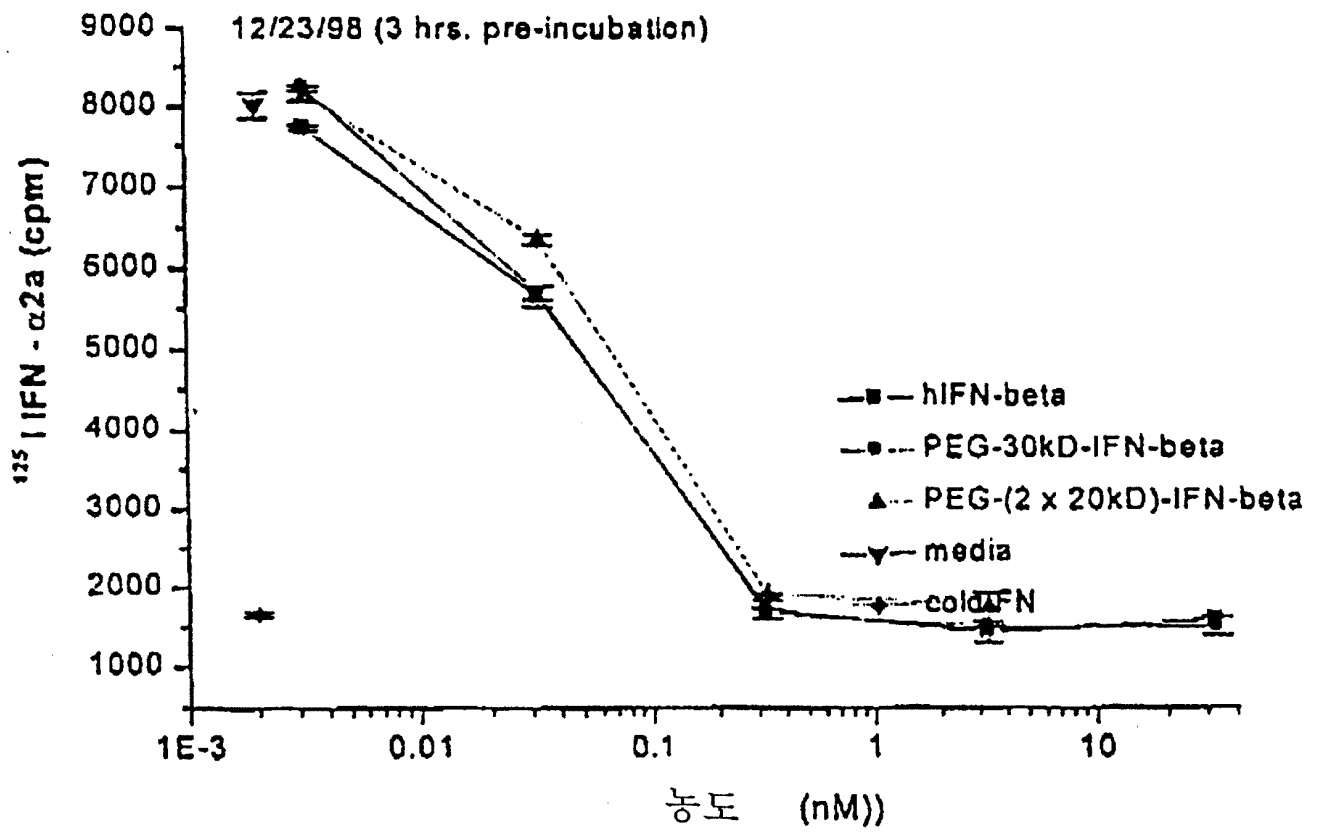
O.D. at 595 nm

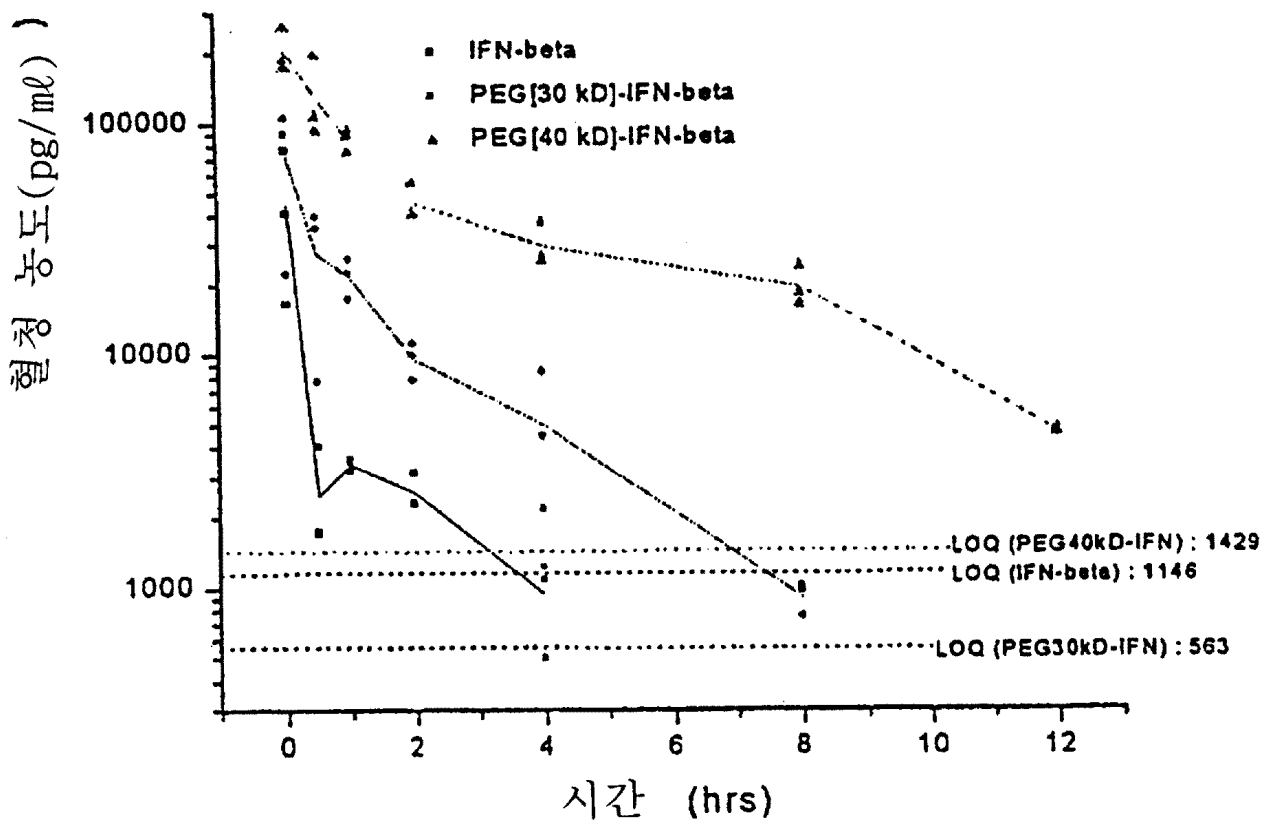


IFN-베타의 농도 (pg/ml)*

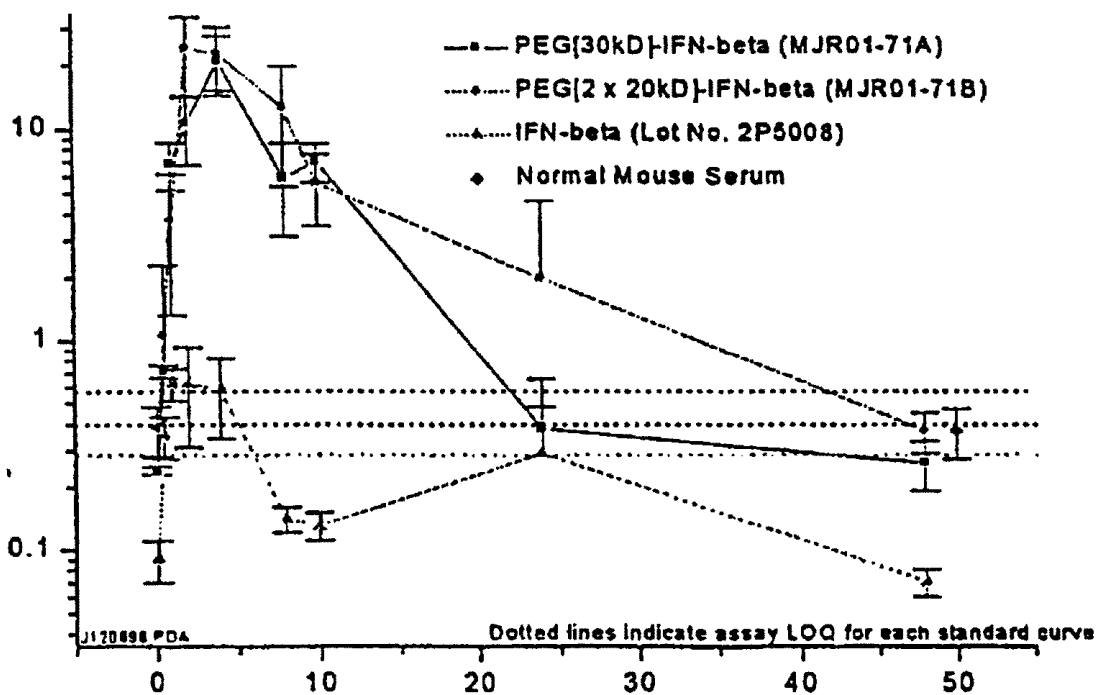
* 아미노산 분석으로 결정한 농도

7





ELISA(Toray)로 결정한 IFN-베타
(또는 PEG-IFN-베타) (ng/ml) 혈청 농도



투여후 시간(피하)