

(19) (12) (KR) (A)

(51) 。 Int. Cl.⁷
C07K 14/56

(11)
(43)

10-2004-0022244
2004 03 11

(21)	10-2004-7002138		
(22)	2004 02 12		
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(86)	PCT/US2002/025691	(87)	WO 2003/016472
(86)	2002 08 12	(87)	2003 02 27

(30) 60/311,866 2001 08 12 (US)

(71) 94502 1265

(72) 94501 2930

94502 14

92064 16515

(74)

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(54) / ,

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- Akiyama, K. et al. (1993) *alpha 1* *alpha 1* . J. Vet. Med. Sci. 55: 3,449-452.
- Ausubel, F. M., et al. (1992) in Current Protocols in Molecular Biology.
- Babiuk, L. A. (1987) *1* *alpha* Antimicrob. Agents Chemother. 31: 5,752-757.
- Bacila et al. eds. (1978) .
- Balzarini, J, et al., Biochem. Biophys. Res. Commun. 178 : 563-569 (1991).
- Bartol, F. F., et al., Biol. Reprod. 33 : 745-759 (1985).
- Bayne, M. L. et al., 66 : 235-244 (1988).
- Bazer, F. W. , and Johnson, H. M., Am. J. Reprod. Immunol. 26 : 19-22 (1991).
- Bazer, F. W., et al., PCT publication WO/94/10313, published 11 May, 1994.
- Beames, et al., Biotechniques 11 : 378 (1991).
- Benvegnu, L., et al., Cancer 83 : 901-909 (1998).
- Berenguer M., et al., Adv. Gastroenterol. Hepatol. Clin. Nutr. 1 : 2-21 (1996).
- Bitter et al. (1984) Proc. Natl. Acad. Sci. 81: 5330-5334.
- Brake, A. J., et al. (1984) *Saccharomyces cerevisiae* Alpha- - Proc. Natl. Acad. Sci. USA 81: 4642-4646.
- Breitling, R. et al. (1989) *alpha* *Escherichia coli* *Baci* *illus subtilis* . Mol. Gen. Genet. 217: 2-3, 384-91.
- Brierley, R. A. (1998) - *1* (IGF-1) . Mol. Biol. 103,149-177.
- Brocca, S., et al. (1998) *Candida rugosa lip1* , , Sci. 7,1415- 1422.
- Cereghino, J. L. and Cregg, J. M. (2000) *Pichia pastors* . FEMS Microbiology Reviews 24,45-66.
- Charlier, M. , et al., Mol. Cell Endocrinol. 76 : 161-171 (1991).
- Cheng, et al. (1997) IFN-*alpha 1* *B* . Chung Kuo Chung Hsi I Chieh Ho Tsa Chih 17: 8, 453-455.
- Choo, Q. -L., et al., Science 244 , 359-362 (1989).
- Choo, Q. -L., et al., Proc. Natl. Acad. Sci. U. S. A. 88 ,2451-2455 (1991).
- Clarke, B. E., Baillieres Best Pract. Res. Clin. Gastroenterol. 14 : 293-305 (2000).

- Cohen et al. (1980) Proc. Natl. Acad. Sci. USA 77: 1078.
- Cotler, S. J., et al., *J. Viral Hepatitis* 7 : 211-217 (2000).
- Crawford-Miksza, L. and David Schnurr, D. (1994)
J. Clinical Microbiology 32 (9): 2331-2334.
- Cregg, J. M. and Madden, K. R. (1989) 가 - . Mol. Gen. Genet. 219,320-323.
- Cregg, J. M. and Russell, K. A. (1998) . Mol. Biol. 103, 27-39.
- Cregg, J. M., et al. (1985) *Pichia pastors* . Mol. Cell. Biol. 5,3376-3385.
- Cregg, J. M., et al. (1988) , *Pichia pastors* . Dev. Ind. Microbiol. 23,33-41.
- Cregg, J. M., et al. (1989) *Pichia pastors* . 2 Mol. Cell. Biol. 5,111-1121.
- Cregg, J. M., et al. (1993) *Pichia pastors* . Bio/Technology 11: 905-910.
- Clare, J. J., et al (1991) : *Pichia pastors* . Gene 105,205-212.
- Cross, J. C. , and Roberts, R. M. , Proc. Natl. Acad. Sci. USA 88 : 3817-3821 (1991). Dayhoff et al. (1978) i n Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found. , Washington, D. C.).
- De Maeyer, E. et al. (1982) alpha 1 . Proc. Natl. Acad. Sci. USA 79: 14,4256-4259.
- Deutscher, (1990) . 182.
- Di Bisceglie, A. M., et al., Hepatology 16 : 649-654 (1992).
- Dieperink, E., et al., Am. J. Psychiatry 157 : 867-876 (2000).
- Ecker, D. J., et al., J. Biol. Chem. 264 : 7715-7719 (1989).
- Elliott et al. (1983) Proc. Natl. Acad. Sci. USA 80: 7080-7084
- Ellis, S. B., et al. (1985) *Pichia pastoris* 2 가 . Mol. Cell. Biol. 9,1316-1323.
- Feher, Z. , et al., Curr. Genet. 16 : 461 (1989).
- Fernandez H., et al., Eur. J. Epidemiol. 2 : 1-14 (1986).
- Godkin, J. D., et al., J. Reprod. Fertil. 65 : 141-150 (1982).
- Gnatek, G. G., et al, Biol. Reprod. 41 : 655-664 (1989).
- Henikoff et al. (1981) Nature 283: 835.
- Higgins, D. R., et al. (1998) . Method Mol. Biol. 103,41-53.

- Hitzeman, R. A., et al., 1988.10.4 U. S. Patent No. 4,775, 622,
- Helmer, S. D., et al., J. Reprod. Fert. 79 : 83-91 (1987).
- Hollenberg et al. (1981) Curr. Topics Microbiol. Immunol. 96: 119.
- Horiike N., et al., C. Oncol. Rep. 5 : 1171-1174 (1998).
- Houglum, Clin. Pharm. 2 : 20-28 (1983).
- Imakawa, K. , et al., Nature 330 : 377-379 (1987).
- Imakawa, K. , et al., Mol. Endocrinol. 3 : 127 (1989).
- Jarpe, M. A., et al., Engineering 7 : 863-867 (1994).
- Jimenez-Saenz, M., et al., J. 15 : 567-569 (2000).
- Jin, X. Y. (1992) rHuIFN alpha-1 . Chung Hua Yen
Ko Tsa Chih 28: 3,134-137.
- Julius et al. (1983) Cell 32: 839-852
- Klemann, S. W., et al., Nuc. Acids Res. 18 : 6724 (1990).
- Koskinas J., et al., J. Med. Virol. 45 : 29-34 (1995).
- Kunkel (1985) Proc. Natl. Acad. Sci. USA 82: 488-492.
- Kunkel et al. (1987) Method Enzymol. 154: 367-382.
- Lechner, F., et al., J. Exp. Med. 191 : 1499-1512 (2000).
- Liu, H., et al. (1992) *Pichia pastors* - .
- J. Bacteriol. 174,4943-4951.
- Liu, H., et al. (1995) PER3, *Pichia pastoris* ,
. J. Biol. Chem. 270,10940-10951.
- Ludwig, D. L., et al., Gene 132 : 33 (1993).
- Magrin, S., et al., Hepatology 19 , 273-279 (1994).
- Maniais T., et al., in MOLECULAR CLONING : A LABORATORY MANUAL , Cold Spring
Harbor Laboratory, Cold Spring Harbor, NY (1982).
- Martal, J. , et al., J. Reprod. Fertil. 56 : 63-73 (1979).
- Martin, E. W. , _____ (Mack Publishing Co., Easton,
PA), 1976.
- Mercereau-Puigalon et al. (1980) Gene 11: 163.
- Mullis, K. B., 1987. 7. 8 U. S. Patent No. 4, 683, 202,

- Mullis, K. B., et al., 1987.7.28 U. S. Patent No. 4,683, 195,
- Noisakran, S. and Carr, D. J. J. (2000) CD4+ CD8+ T¹
IFN- α 1 DNA. *Journal of Immunology* 164 (12): 6435-43.
- Noisakran, S., et al. (1999) IFN- α 1 DNA
1- . *J Immunology* 162 (7): 418490.
- Oeda, K., et al., U. S. Patent No. 4,766, 068, issued August 23,1988.
- Ott, T. L., eta/., J. IFN Res. 11 : 357-364 (1991).
- Panthier et al. (1980) Curr. Genet. 2: 109.
- Pawlotsky, J-M. , et al., *J. Interferon and Cytokine Res.* 15 : 857-862 (1995).
- Pearson, W. R. and Lipman, D. J., *PNAS* 85 : 2444-2448 (1988).
- Pearson, W. R., *Methods in Enzymology* 183 : 63-98 (1990).
- Pontzer C. H., et al. (1995) Measurement of Interferons. Meth. Neurosci. 24: 3-9.
- Raemaekers, R. J. M., et al. (1999) *Pichia pastoris* (PHA)
(GNA): N- 가 PHA-E . Eur
. J. Biochem. 65,394-403.
- Reilly, P. R., et al., BACULOVIRUS EXPRESSION VECTORS : A LABORATORY
MANUAL , 1992.
- Riesenber, D. et al. (1990) alpha 1 *Escherichia coli* . A
ppl. Microbiol. Biotechnol. 34: 1,77-82.
- Roberts, R. M. , et al., Endocrin. Rev. 13 : 432-452 (1992).
- Rose and Harrison, eds. (1987) *The Yeasts* (2nd ed.).
- Rutter, W. J., et al., U. S. Patent No. 4,769, 238, issued September 6, 1988.
- Saito, H., et al., *J. Viral Hepatitis* 7 : 64-74 (2000).
- Sambrook, J., et al. (1989) Molecular Cloning : A Laboratory Manual. Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Scopes, (1982) Protein purification: principles and practice. Springer- Verlag, New York.
- Sears, I. B. , et al. (1998) *Pichia pastoris*
. Yeast 14,783-790.
- Shaw, K. J., et al., DNA 7: 117 (1988).
- Shen, L. P., et al., Sci. Sin. 29 : 856 (1986).
- Shen, S., et al. (1998) *Pichia pastoris* -
. Gene 216,93-102.

- Shepherd, et al. (1998) Kaposi's sarcoma zidovudine 가
alpha 2 : a Canadian HIV Clinical Trials Network Stu
dy. J. Clin. Oncol. 16: 5,1736-1742.
- Shindo, M., et al., Hepatology 9 : 715-719 (1989)
- Singh et al. (1983) Nucleic Acids Res. 11: 4049-4063
- Singh, A. et al. (1984) alpha - , 가 . Nucleic Acids Res. 12:
23,8927-8938.
- Smith et al. (1985) Science 229: 1219-1229.
- Skinner et al., eds. (1980) (Soc. App. Bacteriol. Symp. Series No. 9).
- Stewart, H. J., et al., Mol. Endocrinol. 2 : 65 (1989).
- Strathern et al., eds. (1981) Saccharomyces .
- Thill, G. P., et al. (1990) *Pichia pastors*
In : Proceedings of the Sixth International Symposium on the genetics of Microorganisms (Heslot, H. , e
t al., Eds.), Vol. 2, pp. 477-490. Societe Francaise de Microbiologie, Paris.
- Trepo, C. , *J. Viral Hepatitis* 7 : 250-257 (2000).
- Tyring, et al., Interferon : Principles and Medical Applications, 15t Edition, Section VIII., pgs 399-408, 1992.
- Tschopp, J. F., et al. (1987). *Pichia pastors* 2 - LacZ . Nu
cleic Acids Res. 15,3859-3876.
- Vallet, J. L., et al., Biol. Reprod. 37: 1307 (1987).
- Van Heeke, G., et al. (1996) *Pichia pastors* . *J. Inter
feron and Cytokine Res* . 16: 119-126.
- Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New Yor
k).
- Walter, M. R., et al. (1998) 40 alpha
. Cancer Biotherapy and Radiopharmaceuticals 13: 3,143-154.
- Waterham, H. R., et al. (1996). *Pichia pastors* PER6
Zellweger syndrome PAF-1 가 . Mol. Cell. Biol. 16, 2527-2536.
- Waterham, H. R., et al. (1997) *Pichia pastoris* -3-
. Gene. 186,37-44.
- Whaley, A. E., et al., J. Biol. Chem. 269 : 10864-10868 (1994).
- White, C. E., et al. (1995) , : 6 3
88 . *Protein En g*. 8,1177-1187.
- Wu, D. A., et al., DNA 10 : 201 (1991).

(IFN)

. 1957

, IFN

(Walter, et al., 1998).

IFN () I () IFN- , - , - , - IFN- (II ,) 1 IFN .

[1]

인터페론의 개요

양상	유형 I	유형 I	유형 I	유형 II
타입	α & ω	β	τ	γ
생성의 유래 :	백혈구	섬유아세포	영양아충	림프구
항-바이러스	+	+	+	+
항-증식	+	+	+	+
임신 신호	-	-	+	-

IFN- 2 14 IFN- 12 IFN- IF
N- , 14 80% .

-D(IFN D) -1 (IFN 1) IFN D I (Noisakra
n and Carr, 2000; Noisakran, et al., 1999). IFN D
가 .

1982 IFN D (rHuIFN D) lac *Methylophilus methylotrophus*
E. coli (De Maeyer, 1982). 1984 , ntech -
IFN D 가 가 IFN D *Saccharo*
myces cerevisiae (Singh, 1984). , 1989 *E. coli* *Bacillus subtilis* (*B. subtil*
is) (Breitling,
1989). , *E. coli* *B. subtilis* rHuIFN D
.*E. coli* 1990
 , 11 IFN D *E. coli* rHuIFN D 가 .

IFN- 18-19 kDa IFN- (OvIFN-). 가 가 (
) (Martal, et al., 1979). , (Godkin, et al., 1982).
OvIFN-
-one (oTP-1) . OvIFN-
(Bazer and Johnson, 1991).

, N-
cDNA (Imakawa, et al., 1987) , , IFN- 45-55% IFN- IF
N- II 70% cDNA (St
ewart, et al., 1989; Klemann, et al., 1990; and Charlier, M., et al., 1991). 23 1
72 585 가 1 kb .
4 IFN- I IFN (Jarpe, et al., 199
4).

IFN- I IFN (1), I IFN
 . 가 . IFN-

163-166 - C- - 163-172

가 ,
- ,
1×10⁸ /mg 가
MDBK/VSV - 2×10⁸ /mg 가
MDBK/VSV

P. pastoris

가

- . 1 HVV 1 ;
- . 2 HVV 2 ;
- . 3 HVV 3 ;
- . 4 HVV 4 ;
- . 5A-5C HVV -pPICZ- ;
- . 6A-6B HVV ;
- . 7 1% 72 *P. pastoris* HVV SDS-PAGE ;
- . 8 *P. pastoris* HVV SDS-PAGE .

. 9

PBMC

. 10

I. _____

가 . Sambrook et al.,
1989, Ausubel F M et al., 1993, . , ,

가 .
() .

가 . ' ' /DNA
/DNA

DNA 가
가 .

가 , DNA, DNA, , .

가 가 (ds) DNA .

가 가 가 가 ,

가
(' ')

가
(, 가) . 가 가 가
가 가 ()

가 가 가
DNA

가 DNA 가 ; DNA

가 ; 가 DNA
 , 가
 가
 DNA () () 5'
 (5' UTR) 3' UTR
 가
 ()
 (mRNA) (DNA)
 가
 N-
 / ()
E. coli
 가
 C HCV' -A -B (NANBH)
 RNA HCV RNA
 10^{-3} 10^{-4} HCV
 RNA HCV 가
 /
 'OAS 2',5'- (OAS)
 (Endomycetales), (Blastomycetes)
 Spermophthoraceae Saccharomycetaceae 4
 Schizosaccharomycoideae (, Schizosaccharomyces), Nadsonioideae, Lipomycoideae, Saccharo
 mycoideae (, Pichia, Kluyveromyces, Saccharomyces) Leucosp
 oridium, Rhodosporidium, Sporiobolus, Filobasidium, Filobasidiella
 Sporobolomycetacea (, Sporoholomyces, Bullera) Cryptococcaceae (, Candida)
Pichia, Kluyveromyces, Saccharomyces, Schizosaccharomyces, Cand
 ida. *Pichia* *P. pastoris*
 Skinner et al 가 ,
 , Bacila et al.; R
 ose and Harrison; Strathern et al.;

IFN

DNA

, Walker and Gaastra, eds. (1983); Kunkel (1985); Kunkel et al. (1987); and Sambrook et al. (1989) Dayhoff et al. (1978)

가
Gly Ala, Val Ile Leu, Asp Glu, Lys Arg, Asn Gln, Phe Trp Tyr
IFN 가
DNA
mRNA
IFN/IFN-
80%, 90%, 가
95%
1, 2, 3, 4 IFN
가
IFN
가
1-
가
204,022 IFN U.S. Pat. No. 6,
가
가

II. _____

IFN- C- - I N- 가
가 DNA IFN- C- IFN-
IFN-
10 , 가
163 N- 가
C- C- 5' 163 HVV IFN- D IFN- 1 IFN- D 172 C- 가
N- 3' IFN-
가 IFN-
5 6
IFN- (163-172)
DNA 1-166 IFN- IFN- D IFN- IFN-
10 C- 가 IFN- IFN- IFN-
N- 10 C- 가 IFN- IFN- IFN-
가 IFN- /IFN- IFN- IFN-
N- IFN- D C- IFN-
IFN GenBank
가 IFN-
가 IFN

III. _____

가 - / - . *P. pastoris* -
 / - 가 .
 , ,
 5,942,223; Apr. 14, 1998 Sep. 28, 1999 U.S. Pat. Nos. 5,958,402,; Aug. 24, 1999
 6,204,022; May 25, 1999 5,738,845,; Aug. 17, 1999 5,939,286 ; March 20, 2001
 72,206; 5,906,816, ; May 9, 2000 6,060,450, ; Apr. 16, 2002 6,3
 Jul. 19, 2001 U.S. Ser. Nos. 09/910,406,; May 2, 2002 10/137,1
 27 가 가 .

A. *P. pastoris* _____

- / -
Pichia . *Pichia* *P. pastoris* . *P. past*
oris X-33 .
P. pastoris (Cregg, 1993).
P. pastoris 가
 가 .
P. pastoris (Mut) AOX
 가 (Brierley, 1998; and White et al., 1995).

가
 , Yeast Genetic Stock Center, Department of Biophysics and Medical Physics, University of California (Berkeley, Calif.); the American type Culture Collection (Manassas, Va.); Northern Regional Research Laboratories (Peoria, Ill.); Invitrogen (San Diego, Calif.)

B. _____

가 () .
 가 DNA
 IFN
 . 5A-5C pPICZ -HVV .

i. amp; _____

가

가

,
가 (3') RNA
가 ,

(FLD1), *P. pastoris* 3- (GAP),
(PEX8), YPT1 GTPase

AOX1

a. AOX1

Pichia 가 , AOX1 AOX2 , AOX1
(Tschopp, *et al.*., 1987; Ellis, *et al.*., 1985; and Cregg, *et al.*., 1989). AOX1
AOX1 , poly(A) RN
A 5%가 AOX1 ; , AOX1 가
(Cregg, *et al.*., 1988). AOX1 : *S. cerevisiae* GA
L1 / ,

GAL1 , AOX1 가
(Tschopp, *et al.*., 1987).

b. GAP

P. pastoris 가 AOX1 가 가 *P. pastoris* GA
AP (Waterham, *et al.*., 1997). - *G*
3 2 3 1 . GAP
GAP

c. FLD1

FLD1 -
(Shen, *et al.*., 1998). FLD1
() , AOX1
PFLD1 . FLD1

d. PEX8 YPT1

P. pastoris , AOX1, GAP, FLD1
P AOX1
(Thill, *et al.*., 1990; Brierley, 1998) 가
YPT1 가 , *P. pastoris* PEX8 (Li
u, *et al.*., 1995). . PEX8
YPT1 GTPase
(Sears, *et al.*., 1998).

e. _____

가

RNA

Cohen et al. (1980); Mercereau-Puigalón et al. (1980); Panthier et al. (1980); Henikoff et al. (1981); and Hollenberg et al. (1981)

ii. _____

가 , 가 IFN

N-
(ER)

. ER

가

ER 가 ,

가

Lys Arg Lys-Lys, Lys-Arg, Arg-Lys, Arg-Arg, 가 Lys-Arg

). KEX2 *P. pastoris* 가 KEX2 (Julius et al. (1983
(, , Sambrook et al. (1989)).

. 가 ,

3'
ER

가

a. *S. cerevisiae* - _____

, Singh et al. (1983)
, Elliott et al. (1983); Bitter et al. (1984); Smith et al. (1985).

가 11 - , 가 100 200 , 120
-160 () 19-23 (20-2
2), 60-66 (), 2-6
가

- 3', Kex2 Glu-Ala pro Arg-Lys (Brake, et al, 1984). pre Ste13
- 3' N- pro 5' IFN 가
- 5'
- 가
- b. _____
- PHA-E *Phaseolus vulgaris* *P. pastoris* (PHO1) (Cereghino and Cregg, 2000; and Raemaekers, et al., 1999). 가 , cDNA (Sambrook et al. (1989)).
- IFN D
- iii. _____ IFN
- 1-4 IFN 2-HVV 1
- 가 IFN 가
- 가 U.S. Pat. Nos. 5,219,759 5,602,034).
- 가
- G-C
- mRNA
- iv. _____
- mRNA
- Mat- (-)
- AOX1
- v. _____ 가
- 가 *P. pastoris* *S. cerevisiae*, HIS4, *S. cerevisiae* ARG4, *Streptoalloteichus hindustanus* Sh ble (Cregg et al., 1985; Cregg and Madden, 1989; and Higgins, et al., 1998)

P. pastoris ADE1 (PR-,), ARG4 (),
 URA3 (5'-) .

vi. _____

IFN ,

가

Sambrook et al. (1989)

가 DNA (, ,)

가 , 가 (Clare, et al., 1991).

B. _____

(Cregg et al., 1985; Liu et al., 1992; Waterham et al., 1996; and Cregg and Russell, 1998).

C. _____ IFN

DNA ,

가 3 IFN , HVV 2 . HVV

3×10^{-8} U/mg , *P. pastoris* IFN 4 2.75×10^{-8} U/mg

5 가 Pontzer and Johnson, 1985

D. _____ IFN D

Pichia pastoris rHuIFN D *Pichia* 가

Pichia rHuIFN D 가

er, 1990 and Scopes, 1982 Deutsch

(Fernandez, et al., 1986; Balzarini, et al., 1991).
C HCV RNA 가 (Di Bisceglie, et al., 1992).
(Clarke, 2000; Koskinas, et al., 1995)
, IFN- (Cotler, et al., 2000). IF
N IFN-
가
가
가

[2]

본 발명에 따라 제공된 서열

설명	SEQ. ID NO.
<u>HVV 아미노산 서열</u> CDLPETHSLDNRRITMLLAQMSRISPSSCLMDRHDFGFPQEEFDGN QFQKAPAIISVLHELIQQIFNLFTTKDSSAAWDEDLLDKFCTELYQQLN DLEACVMQEERVGETPLMNADSLAVKKYFRRITLYLTEKKYSPCAWE VVRAEIMRSLSLSTNLQERLTKMGGDLNSP	1
<u>HVV 핵산 서열</u> TGTGATTTGCCAGAGACTCACTCTTTGGACAACAGAAGAACTTTGA TGCTTTTGGCCCAAATGTCTAGAATCTCTCCATCCTCTTGTGTTGAT GGATAGACACGATTTGCGTTTCCCACAAGAAGAAATTTGACGGTAA CCAATTCCAAAAGGCTCCTGCTATTTCTGTTTTGCACGAGTTGATT CAACAAATTTCAACTTGTTCACTAAGGACTCTTCTGCTGCCT GGGACGAAGACTTGTTGGACAAGTTCTGTACTGAGCTTTACCAAC AATTGAACGACTTGAGGCTTGTGTTATGCAAGAGGAGAGAGTCG GTGAGACCCCATTTGATGAACGCTGATTCCATCTTGGCTGTCAAGA AGTACTTCAGAAGAATTACCTTGTACTTGACCGAAAAGAAGTACTC CCCATGTGCCTGGGAAGTCGTTAGAGCCGAAATCATGAGATCTTT GTCCCTTGCCACTAACTTGCAAGAGAGACTTACCAAGATGGGTGG	2

AGACTTGAAGTCTCCATAA

VII. _____

1

HVV _____

HVV C- 4 IFN- 10 6 C- H
uIFN D 가 DNA , *Pichia pastoris* (가 150

; 1-4) G2
Technologies, Alameda, Calif.).

DNA

(Operon

(가 40 bp) 4 150 5' 3' (HVV 1-4) .

A. _____ #1

:G2

:XbaI BamHI

: 1 (. 1)

:G2HV1F1

B. _____ #2

:G2HV1F1

:EcoRI BamHI

: 2 (. 2)

:G2HV1F2

C. _____ #3

:G2HV1F2

:Sac I BamHI

: 3 (. 3)

:G2HV1F3

D. _____ #4

:G2HV1F3

:EcoRI BamHI

: 4 (. 4)

: G2HV1F4

E. _____

: Sac II

XhoI NotI HVV
vitrogen, San Diego) . HVV -pPICZ -

G2HV1F4
. 5A-5.

pPICZ - (In
.

2

P. pastoris _____ HVV

P.pastoris HVV BMGY , BMMY
 2 OD₆₀₀ 24 48 10% 1M 가
 72 , , Colloidal Coomassie (Novex, San Diego, Calif
 .) 14% Tris-glycine SDS-PAGE . pPICZ
 . .7 , HVV-16 HVV 가 .

3

HVV _____

. 8 *P.pastoris* HVV (20M) 10 Tris,
 NaCl 150 HiPrep Sephacryl 26/60 S-100 - 2
 M (pre-) 120 M 1 M/min 1
 . 20 M 2, 3, 4 2, 3 4 1 M 1
 4% SDS-PAGE Novex colloidal blue .

4

HVV - _____

3 HVV - HVV 16(. 7) *P. past*
oris (IDs: S-0018; S-0019; S-0
 063; S-0064) 3(. 8) 1 M
 5 3 .

[3]

HVV-16 항-바이러스 특이적 활성

표본 ID	단백질 농도 (MG/ML)	항-바이러스 단위 (AVU/ML)	항-바이러스 특이적 활성 (AVU/MG)	평균 AVU/MG ± STD (N)
S-0018	0.751	2.22 x 10 ⁸	2.96 x 10 ⁸	2.86 x 10 ⁸ ± 0.09 (N = 4)
S-0019	0.696	1.92 x 10 ⁸	2.76 x 10 ⁸	
S-0063	1.102	3.11 x 10 ⁸	2.82 x 10 ⁸	
S-0064	1.422	4.10 x 10 ⁸	2.88 x 10 ⁸	

5

5

Madin Darby bovine kidney(MDBK) 96- 10% fetal serum(FBS)
 Eagle's MEM 가 , 2%FBS
 Eagle's MEM (16-24) MDBK 100% CPE
 VSV . 96-
 200μl/ 4 VSV 50μl/ 10⁻¹ 10⁻⁶
 . 100% CPE , TCID50 ,
 10^{6.5} TCID₅₀ /M 가 10^{3.5} TCID
 50 / .
 , 가 PBS MDBK

100
 μl 10 (10^{-1} 10^{-10}) 3 가 18
 37 HulFN A(Biosource Intl., Camarillo, CA)
 @ $10^{3.5}$ TCID₅₀ / 100 μl 가
 PBS 가 16-24
 37 100 μl 0.2% neutral red solution (Gibco-BRL) 100 μl
 37 1 100 μl (50% , 1%) 가
 가 PBS 2 A₅₅₀ Bio-Kinetics Reader (Bio-Tek In
 struments, Winooski Vt.).

$$\text{보호퍼센트} = \frac{AVG(A550\text{시험웰}) - AVG(A550\text{바이러스대조구웰})}{AVG(A550\text{비처리된세포대조구웰})} \times 100$$

1 - (U) 50% (/M ℓ) 50%
 (0.1 M ℓ) 10
 10- /M ℓ (
 10)
 - /M ℓ Crawford-Mikszs (1994)가

6

IFN

(PBMC) HulFN , OvIFN , IFN
 PBS 1:4 Nycoprep 1.077 (Nycomed Pharma, Oslo, Nor
 way). 20 20 600xg
 IFN , IFN , 96-
 IFN 5x10 /
 2000 U/M ℓ 128,000 U/M ℓ 3
 가 (Pharmingen). 12

V :

V (PS) 35-36 kD Ca V , PS 가 V

PBMC 3 Pharmingen V V
 Becton dickinson FACScan Quest Software
 PBMC (V ,)

V+ 10,000 (

$$\text{특이적세포죽음\%} = \frac{\text{처리되지않은웰유래의아넥신I*양성세포\%}}{\text{처리된웰유래의아넥신I*양성세포\%}} \times 100$$

* : % V

7

1.5×10⁵ - U/Mø rHuIFN roIFN PBMC (5×10⁵ /) 12
V-FITC
: [(% AV+ PI+)/(% AV+ PI+)×100].

[4]

애넥신 V 양성 세포에 근거한 특이적 세포 죽음의 퍼센트

	HVV-16	rHuIFNαD (Biosource)	rHuIFNαD (Pepgen)	BSA 대조구
AVG ± STD	12.88 ± 15.44	65.00 ± 22.49	45.46 ± 23.64	7.67 ± 14.57
중양값	4.88	65.78	43.95	0.00
N	6	10	10	10

[5]

프로피디움 아이오다이드 양성 세포에 근거한 특이적 세포 죽음의 퍼센트

	HVV-16	rHuIFNαD (Biosource)	rHuIFNαD (Pepgen)	BSA 대조구
AVG ± STD	2.08 ± 3.85	32.88 ± 15.69	19.32 ± 12.55	3.78 ± 8.19
중간값	0.56	34.59	15.27	0.00
N	6	10	9	10

8

9 ×10⁵ - U/Mø rHuIFN roIFN PBMC (5×10⁵ /) 9, 10, 12 1.5
V-FITC
: [(% AV+ PI+)/(% AV+ PI+)×100].

(57)

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	C-	-	C-	,	
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	7.				
6	,	C-	1-10	가	
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	8.				
7	,	C-	4	가	
	.				
	9.				
5	,	- D	C-	163-166	
	.				
	10.				
1	,	- C-	-	163-172	
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	11.				
1	,	- D	- D	C-	
163-166	,	- C-	-	163-172	
	.				
	12.				
1	,			가	
	.				
	13.				
1	,	MDBK/VSV		1×10^{-8}	
/mg	가				
	14.				
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	15.				
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1

HVV 단편1

합성된 올리고의 목록:

HVV1F1T1 5'-PCTAGGCTCGAGAA-3'
 HVV1F1T2 5'-PGAGATGTGATTGCCAGAGACTCACTCTT-3'
 HVV1F1T3 5'-PTGGACAACAGAAGAAGTTTGATGCTTTT-3'
 HVV1F1T4 5'-PGGCCAAATGTCTAGAATCTCTCCATCCTC-3'
 HVV1F1T5 5'-PTTGTGATGGATAGACACGATTTCGGTTT-3'
 HVV1F1T6 5'-PCCCAACAAGAAGATTCCCTGCTCGCG-3'
 HVV1F1B1 5'-PGATCCGCGAGCA-3'
 HVV1F1B2 5'-PGGGAATTCTTCTTGTGGGAAACCGAAATCGT-3'
 HVV1F1B3 5'-PGTCTATCCATCAAACAAGAGGATGGAGA-3'
 HVV1F1B4 5'-PGATTCTAGACATTGGGCCAAAAGCATCA-3'
 HVV1F1B5 5'-PAAGTTCTTCTGTTGTCCAAAGAGTGAGTCT-3'
 HVV1F1B6 5'-PCTGGCAAATCACATCTCTTCTCGAGC-3'

최종 연결된 산물:

{ HVV1F1T1 } { HVV1F1T2 } { HVV1F1T3 }
 5'-CTAGGCTCGAGAAGAGATGTGATTGCCAGAGACTCACTCTTGGACAACAGAAGAAGTT
 3'-CGAGCTCTTCTTACACTAAACGGTCTCTGAGTGAGAAACCTGTTGTCTTCTTGAA
 { HVV1F1B6 } { HVV1F1B5 }
 { HVV1F1T4 } { HVV1F1T5 }
 TTTGGCCCAATGTCTAGAATCTCTCCATCCTTGTGTTGATGGATAGACACGATTTCGGTTT
 AAACCGGGTTTACAGATCTTAGAGAGGTAGGAGAACAACCTACCTATCTGTGCTAAAGCCAAA
 F1B4 { HVV1F1B3 } { HVV1F1B2 }
 1T6 }
 GAAGAATTCCTGCTCGCG-3'
 CTCTTAAGGACGAGCGCCTAG-5'
 { HVV1F1B1 }

2

HVV 단편 2

합성된 올리고의 서열:

HVV1F2T1 5'-PAATTGACGGTAAC-3'
 HVV1F2T2 5'-PCAATTCAAAAGGCTCCTGCTATTTCTGT-3'
 HVV1F2T3 5'-PTTTCACAGAGTTGATCAACAAATTT-3'
 HVV1F2T4 5'-PTCAACTTGTTCAACCTAAGGACTCTT-3'
 HVV1F2T5 5'-PCTGCTGCCTGGGACGAAGACTTGTGGAC-3'
 HVV1F2T6 5'-PAAGTTCTGTACTGAGCTCAGCGCAATG-3'
 HVV1F2B1 5'-PGATCCATTTCGCGCT-3'
 HVV1F2B2 5'-PGAGCTCAGTACAGAAGTTGTCCAACAA-3'
 HVV1F2B3 5'-PGTCTTCGTCCAGGACGAGAGAGTCT-3'
 HVV1F2B4 5'-PTAGTGGTGAACAAGTTGAAAATTTGTG-3'
 HVV1F2B5 5'-PAATCAACTCGTGCAAAACAGAAATA-3'
 HVV1F2B6 5'-PGCAGGAGCCTTTTGGAAATTGGTTACCGTCA-3'

최종 연결된 산물:

{ HVV1F2T1 } { HVV1F2T2 } { HVV1F2T3 }
 5'-AATTGACGGTAACCAATTCCAAAAGGCTCCTGCTATTTCTGTTTGCACGAGTTGAT
 3'-ACTGCCATTGGTTAAGTTTTCGAGGACGATAAAGACAAAACGTGCTCAACTA
 { HVV1F2B6 } { HVV1F2B5 }
 { HVV1F2T4 } { HVV1F2T5 } { H
 TTTCAACTTGTTCAACCTAAGGACTCTTCTGCTGCCTGGGACGAAGACTTGTGGACAAG
 AAAGTTGAACAAGTGGTGATTCTGAGAAGACGACGACCTGCTTCTGAACAACCTGTT
 2B4 { HVV1F2B3 } { HVV1F2B2 }
 }
 GAGCTCAGCGCAATG-3'
 CTCGAGTCGCGCTTACCTAG-5'
 { HVV1F2B1 }

3

HVV 단편3

합성된 올리고의 목록:

HVV1F3T1 5'-PTTACCAACAATT-3'
 HVV1F3T2 5'-PGAACGACTTGGAGGCTTGTGTTATGC-3'
 HVV1F3T3 5'-PAAGAGGAGAGAGTCGGTGAGACCCATTGATG-3'
 HVV1F3T4 5'-PAACGCTGATTCCATCTTGGCTGTCAAGAAGT-3'
 HVV1F3T5 5'-PACTTCAGAAGAATTCAGCTCATTG-3'
 HVV1F3B1 5'-PGATCCAAATGAGC-3'
 HVV1F3B2 5'-PTGAATTCTTCTGAAGTACTTCTTGACAGCCAA-3'
 HVV1F3B3 5'-PGATGGAATCAGCGTTCATCAATGGGGT-3'
 HVV1F3B4 5'-PCTCACCAGACTCTCTCCTCTTGCCATAACACA-3'
 HVV1F3B5 5'-PAGCCTCCAAGTCGTTCAATTGTTGGTAAAGCT-3'

최종 연결된 산물:

{ HVV1F3T1 } { HVV1F3T2 } { HVV1F3T3 }
 5'-TTACCAACAATTGAACGACTTGGAGGCTTGTGTTATGCAAGAGGAGAGAGTCGGTG-
 3'-TCGAAATGGTGTAACTTGTGTAACCTCCGAACACAATACGTTCTCTCTCTCAGCCAC-
 { HVV1F3B5 } { HVV1F3B4 }
 { HVV1F3T4 } { HVV1F3T5 }
 ATTGATGAACGCTGATTCCATCTTGGCTGTCAAGAAGTACTTCAGAAGAATTCAGCTCATTG-
 TAACTACTTGGCACTAAGGTAGAACCGACAGTTCTTCATGAAGTCTTCTTAAGTCGAGTAAAC-
 V1F3B3 { HVV1F3B2 } { HVV1F3B1 }

4

합성된 올리고의 목록:

HVV1F4T1 5'-PAATTACCTTGTACT-3'
 HVV1F4T2 5'-PTGACCGAAAAGAAGTACTCCCCATGTGCC-3'
 HVV1F4T3 5'-PTGGGAAGTCGTTAGAGCCGAAATCAT-3'
 HVV1F4T4 5'-PGAGATCTTTGTCCTTGTCCACTAATT-3'
 HVV1F4T5 5'-PGCAAGAGAGACTTACCAAGATGGGTGGAG-3'
 HVV1F4T6 5'-PACTTGAACCTCCATAAGCGGCCGCG-3'
 HVV1F4B1 5'-PGATCCGCGGCCGCT-3'
 HVV1F4B2 5'-PTATGGAGAGTTCAGTCTCCACCCAT-3'
 HVV1F4B3 5'-PCTTGGTAAGTCTCTCTTGCAAGTTAGTGG-3'
 HVV1F4B4 5'-PACAAGGACAAAGATCTCATGATTTCGGC-3'
 HVV1F4B5 5'-PTCTAACGACTTCCAGGCACATGGGGAGTA-3'
 HVV1F4B6 5'-PCTTCTTTTCGGTCAAGTACAAGGT-3'

최종 연결된 산물:

{ HVV1F4T1 } { HVV1F4T2 } { HVV1F4T3 }
 5'-AATTACCTTGTACTTGAACGAAAAGAAGTACTCCCCATGTGCCTGGGAAGTCGTTAGAG-
 3'-TGGAACATGAAGTGGCTTTTCTTCATGAGGGGTACACGGACCCCTCAGCAATCTCC-
 { HVV1F4B6 } { HVV1F4B5 }
 { HVV1F4T4 } { HVV1F4T5 } { HVV1F4T6 }
 ATGAGATCTTTGTCCTTGTCCACTAATTGCAAGAGAGACTTACCAAGATGGGTGGAGACTTC-
 TACTCTAGAAACAGGAACAGGTGATTGAACGTTCTCTCTGAATGGTTCTACCCACCTCTGAAC-
 B4 { HVV1F4B3 } { HVV1F4B2 }
 {
 CATAAGCGGCCGCG-3'
 GTATTGCGCCGGCGCCTAG-5'
 { HVV1F4B1 }

5A

Bgl II Not I
 CGAAATCATGAGATCTTTGCTCTGCTCACTAAGTTCAGACAGAGTATCAGATGGTGGAGACTTGAACCTCCATAACGGCCGACCTT 1728
 GCTTTAGTACTCTAGAAACAGGAACAGGTGATTGAACGTTCTCTCAATGGTTTACCCACCTCTGAACCTTGAGAGGTAATCGCCGGCTCGAA
 E I M R S L S L S T N L O E R L T K M G G D L N S P A A A S F
 Xba I Mbo II
 TCTAGAACAAAACTCATCTCAGAACAGGATCGAATAGCCGCTCGACCATCATCATCATCATTTGAGTTTGTAGCCTTACACATGACTGTTT 1824
 AGATCTTGTGTTTGTAGTAGAGTCTTCTCTAGACTTATCGCGCAGCTGGTAGTAGTAGTAGTAACCTCAACATCGGAATCTGTACTGACAAG
 L E O K L I S E E O L N S A V D H H H H H V C S L R H D C S
 Mbo II
 CTCAGTTCAAGTTGGGCACTTACGAGAAGACCGGTCTTCTAGATTCTAATCAAGAGGATGTCAGAATGCCATTTGCCTGAGAGATGACGGCTTCA 1920
 GAGTCAAGTTCAACCCGTAATGCTCTTCTGCGCAGAACGATCTAAGATTAGTTCTCTACAGTCTTACGGTAAACGGACTCTCTACGTCGGAAGT
 S V Q V G H L R E D R S C I L I K R M S E C H L P E R C R L H
 Mbo II
 TTTTGTACTTTTTATTGTAACCTATATAGTATAGCATTTTTTGTGCTATTTTGTCTTCTCTGACGAGCTTGTCTGATCAGCCTATCTC 2016
 AAAAATCATGAAAAATAAACATTGGATATATCATATCTAAAAAAACAGTAAAAACAAGAGAGCATGCTGAAACGAGGACTAGTCGGATAGAG
 F Y F F I C N L Y S I G F F L S F C F F S Y E L A P D O P I S
 Tse I Mbo II
 GCAGCTGATGAATATCTTGTGCTAGCGGTTTGGCAAAATCATTCGAGTTTCATGTTTTCTTGGTATTTCCCACTCTCTTCAGAGTACAGAAGAT 2112
 CGTCGACTACTTATAGAACACCATCCCAAAACCTTTTAGTAAGCTCAAACTACAAAAGAACCATAAAGGGTGAGGAGAAGTCTCATGCTTCTA
 Q L M N I L W G F G K I I R V C F S W Y F P L L F R V O K I
 Mbo II BamH I Mbo II
 TAAGTGAGACCTTCTGTTGTGGGATCCCCACACCATAGCTTCAAAATGTTTCTACTCTTTTACTCTTCCAGATTTTCTCGGACTCCGG 2208
 ATTCACCTCTGGAAGCAACACGCTAGGGGCTGTGTGTATCGAAGTTTACAAAGATGAGGAAAAATGAGAAGGTCTAAAGAGCCTGAGGCGC
 K D L R L C G S P T H S F K M F L L L F Y S S R F S R T P R
 Mbo II
 CATCGCGTACCCTTCAAAACCCCAAGCAGCAGCATAAATTTCCCTCTTTCTTCTCTAGGGTGTGCTTAATTACCGTACTAAAGGTTTG 2304
 GTAGCGGCTAGGTGAAGTTTGTGGGTGCTGCTATGATTAAAGGGAGAAAGAGAGATCCACAGCAATTAATGGGATGATTCCAAAC
 I A V P L O N T O A O H T K F S L F L P L G C R L P V L K V W
 Mbo II
 GAAAGCAAAAAAGAGACCGCTCGTTTCTTTCTCTGTCGAAAAAGCAATAAAAAATTTATCAGTTTCTTTTCTGAAATTTTTTTTTTA 2400
 CTTTCTTTTCTCTGCGGAGCAAAAGAAAGAGCAGCTTTTCCGTTATTTTAAAAATAGTCAAGAAAAAGAACTTTAAAAAAAAT
 K R K K R P P R F F F F V E K G N K N F Y H V S F S N F F F

5B

Mbo II

TTTCTTCTTTTCAGTCACCTCCATTGATATTTAAGTTAATAAAAGTTTCAATTTTCAGTTTCAGTTTCATTTTCTTGTCTATTACAA 2496
 TAAAAAGAGAAAGTCACCTGGAGTAACATAAATTCATTATTGCCAGAAAGTTAAAGAGTTCAAAGTCAAAGTAAAAAGAACAAAGATAATGTT
 F F S L S V T S I D I V N K R S S I S O V S V S F F L F Y Y N
 TTTTCTACTTCTTGTTCATTAGAAAGAAAGCATAGCAATCTAATCTAAGCGCGGTGTTGACAAITTAATCAICCGCATAGTATATCGGCATAGT 2592
 TAAAAAATGAAGACAAAGTAATCTTTCTTCGTATCGTTAGATTAGATTCCCGCCACAACGTGTTAATTAGTAGCCGTATCATATAGCCGTATCA
 F F Y F L F I R K K A Q S N L R G G V D N S S A Y I G I V
 ATAATACGACAAGGTGAGGAACATAACCATGGCCAAAGTTGACCACTGCCGTTCCGGTGCTCACCBCGCGGAGCTGCCGAGCGGTGAGTTCTG 2688
 TATTATGCTGTTCCACTCTTTGATTGGTACCGGTTCACTGGTACGGCAAGGCCACAGTGGCGCGGCTGCAGCGGCTCCGCCAGCTCAAGAC
 Y D K V R N T M A K L T S A V P V L T A R D V A G A V E F W
 Sma I
 GACCGACCGGCTCGGGTCTCCCGGACTTCGTGGAGGAGCTTCGCGGTGTGGTCCGGACGACGTGACCTGTTTCATCAGCGGGTCCAGGA 2784
 CTGGCTGGCCGAGCCCAAGAGGCGCTGAAGCACCTCTGCTGAAGCGGCCACACCGAGGCTGCTGCACTGGGACAAGTAGTCGCGCCAGGTCCT
 T D R L G F S R D F V E D D F A G V V R D D V T L F I S A V O D
 CCAGGTGCTGCCGACAACACCTGGCTGGGTGTGGGTGCGCGGCTGGACGAGCTGTACGCCGAGTGGTCCGAGGTGCTGTCCAGCAACTTCCG 2880
 GGTCCACCCAGCGGCTGTTGTGGGACCGGACCCACACCCACGCGCGGACTGCTCGACATGCGGCTCACCAGCTCCAGCACAGGTGCTTGAAGGC
 Q V V P D N T L A W V W V R G L D E L Y A E W S E V V S T N F R
 Hga I Tse I
 GGAGGCTCCCGGCGGCGATGACCGAGATCGGCGAGCAGCCGTGGGGCGGGAGTTGCGCTGCGCGAGCCCGCGGCAACTGCGTGCACCTTCGT 2976
 CCTGCGAGGCGCGGCGGTACTGCTCTAGCGCTGCTCGGCACCCCGGCTCAAGCGGAGCGGCTGGCGCGGCGTTGACGCACTGAAGCA
 D A S C P A M T E I G E Q P W G R E F A L R D P A G N C V H F V
 GGCGAGGAGCAGGACTGACAGTCCGACGCGGCGCCACGGGTCCAGGCTCCGAGATCCGTCCCGCTTTCTTTGTCGATATCATGTAATTAG 3072
 CCGGCTCTCTGCTGCTGACTGTGAGGCTGCGCGGGTGCCAGGCTCCGAGGCTCTAGGCGAGGGGAAAGGAAACAGCTATAGTACATTAATC
 A E E Q D H V R R R P T G P R P R R S V P L F L C R Y H V I S
 TTATGTCAGCTTACATTACGCGCTCCCGCCACATCCGCTTAACCGAAAAGGAAGGAGTTAGACAACCTGAAGTCTAGGTCCCTATTATTTTT 3168
 AATACAGTCCGAATGTAAGTGGGAGGGGGGTGAGGCGAGATTGGCTTTTCTTCTCAATCTGTTGGACTTCAGATCCAGGGATAAATAAAAA
 Y V T L T F T P S P H I R S N R K G R S T T S L G P Y L F F
 Mlu I Hga I
 TTATAGTTATGTTAGTATTAAAGCGTTATTTATATTCAAAATTTTCTTTTTTCTGTACAGACGCGGTGACGATGTAACATTATACTGAAAA 3264
 AATATCAATACAATCATATCTTTCATAAATAAAGTTTAAAAAGAAAAAAGACATGCTGCGCACATGCGTACATTGTAATATGACTTTT
 Y S Y V S I K N V I Y I S N F S F F S V Q T R V R H H Y T E N

5C

Friday, July 6, 2001 5:20 PM

HVV+pPICZ-alphaB Map (1 > 4051) 부위와 서열

Hga I

CCTTGCTTGAGAAGGTTTGGGACGCTCGAAGGCTTTAATTGCAAGCTGGAGACCAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA
 GGAACGAACCTCTCCAAACCCCTGCGAGCTTCCGAAATTAACGTTGACCTCTGGTTGTACACTCGTTTTCCGGTCGTTTTCCGGTCTCTGGCAT 3360

L A . E G F G T L E G F N L O A G D O H Y S K R P A K G O E P .

Hga I

AAAAGCCGCGTTGCTGGCGTTTTTCATAGGCTCCGCCCCCTGACGAGCATCAGCAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAG
 TTTTCCGGCGCAACGACCGCAAAAGGATCCGAGCGGGGGAGCTGCTGTAGTGTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTC 3456

K G R V A G V F P . A P P P . R A S O K S T L K S E V A K P D R

GACTATAAGATACCAAGCGTTTCCCTGGAAGCTCCCTGCTGGCTCTCCTGTTTCGACCTGCGCTTACCGGATACCTGTCCGCTTTCTCC
 CTGATATTTCTATGGTCCGCAAAAGGGGACCTTCGAGGGAGCACGCGAGAGGACAAGGCTGGGACGGGAATGGCTATGGACAGCGGAAAGAGG 3552

T I K I P G V S P W K L P R A L S C S D P A A Y R I P V R L S P

CTTCGGGAAGCGTGGCGCTTTCTCAATGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGTCTGCTTCCAGCTGGGCTGTGCGACGAACCC
 GAAGCCCTTCGACCGCAAGAGTTACGAGTGGGACATCCATAGAGTCAAGCCACATCCAGCAAGCGAGGTTGACCCGACACAGTGTCTGGG 3648

F G K R G A F S M L T L . V S O F G V G R S L O A G L C A R T P

Tse I

CCGTTGAGCCGAGCGCTGGCGCTTATCCGGTAAGTATGCTCTGAGTCCAAACCCGTAAGACAGGACTTATCGCCACTGGGAGCAGCCACTGGTA
 GGCAGTTCGGCTGGGACGCGGAATAGGCCATGATAGCAGAACTCAGGTTGGGCCATTCTGTGCTGAATAGCGGTACCGTCTGCTGGTACCAT 3744

R S A R P L R L I R . L S S . V D P G K T R L I A T G S S H W .

ACAGGATTAGCAGAGCGAGGTATGTAGGCGGTCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCT
 TGTCTAATCTGCTCGCTCCATACATCCGCCAGATGTCTCAAGAACTTCACCAACCGGATGATGCGATGTGATCTTCTGTCTATAAACCATAGA 3840

O D . O S E V C R R C Y R V L E V V A . L R L H . K D S I W Y L

GGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACCAACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCA
 CGCGAGACGACTTCGGTCAATGGAAGCCTTTTCTCAACCATCGAGAAGTAGGCCGTTTGTGTTGGTGGGACCATCGCCACCAAAAAACAAACGT 3936

R S A E A S Y L R K K S W . L L I R O T N H R W . R W F F C L O

Tse I

Mbo II

Hga I

AGCAGCAGATTACCGCGAGAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTCTGACGCTCAGTGGAAAGAACTCACGTTAAG
 TCGTCGCTAATGCGCGCTTTTTTTCTAGAGTCTTCTAGGAAACTAGAAAGATGCCCGAGCTGCGAGTCACTTGTCTTTGAGTCAATTC 4032

A A D Y A O K K R I S R R S F D L F Y G V . R S V E R K L T L R

GGATTTTGGTCATGAGATC 4051
 CCTAAAACCACTACTCTAG

D F G H E I

6A

HVJ 단백질 정보

HVJ 아미노산 서열

CDLPETHSLDNRRITMLLAQMSRISPSSCLMDRHDFGFPQEEFDGNQFQKAPAIISVLHELII
QIFNLFTTKDSSAAWDEDLDDKFCETELYQQLNDLEACVMQEERVGETPLMNADSLAVKI
YFRRLITLYLTEKKYSPCAWEVVRAEIMRSLSLSTNLQERLTKMGDLNSP.

단백질 정보

분자량 19816.67 달톤
172 아미노산
18 강한 염기성(+) 아미노산(K, R)
26 강한 산성(-) 아미노산(D, E)
56 소수성 아미노산 (A, I, L, F, W, V)
50 극성 아미노산(N, C, Q, S, T, Y)
4.800 등전점
PH 7.0 에서 7.714 전하

번역된 염기의 전체 수는 519이다.

% A = 27.36 [142]
% G = 21.97 [114]
% T = 28.90 [150]
% C = 21.77 [113]
분류하지 않은 % = 0.00 [0]

% A+T = 56.26 [292]
% C+G = 43.74 [227]

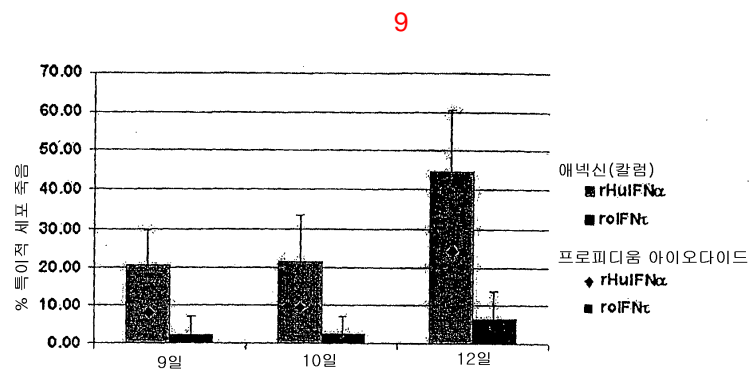
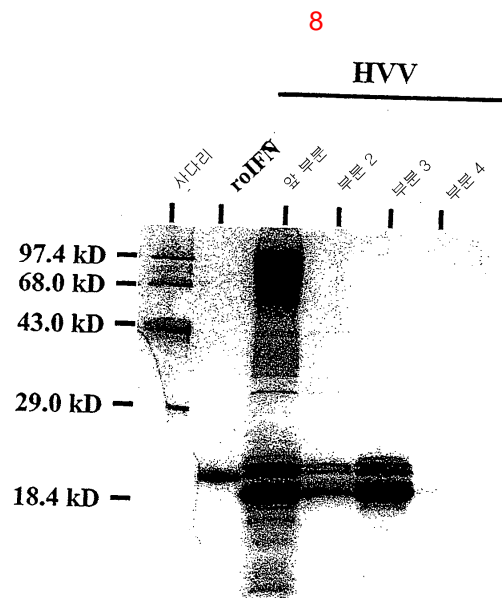
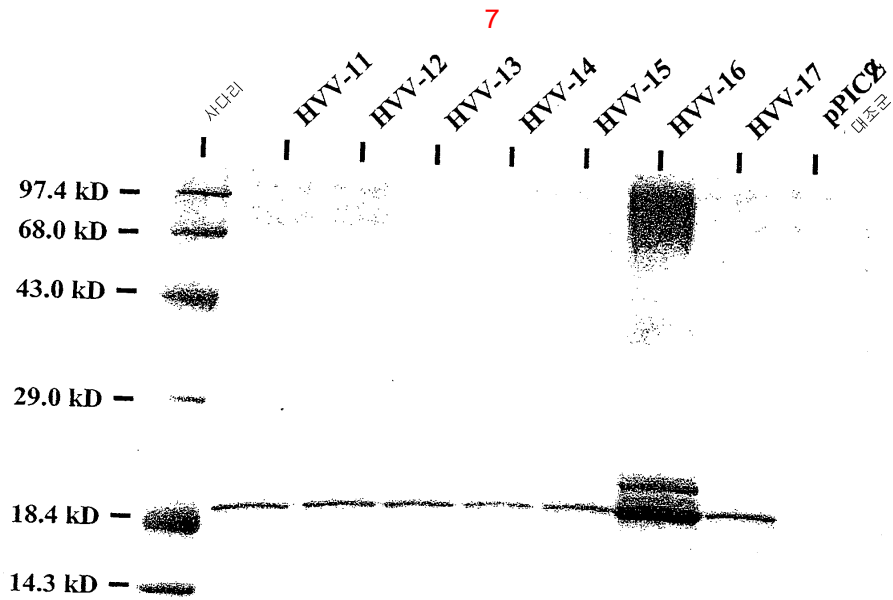
Davis, Botstein, Roth 녹는점 C. 81.87
Wallace Temp C 1718.00

코돈 사용

gca	Ala (A)	0	#	cag	Gln (Q)	0	#	uug	Leu (L)	20	#	uua	Ter (.)	1
gcc	Ala (A)	4	#	---	Gln (Q)	10	#	---	Leu (L)	23	#	uag	Ter (.)	0
gcg	Ala (A)	0	#	gaa	Glu (E)	6	#	aaa	Lys (K)	0	#	uga	Ter (.)	0
gcu	Ala (A)	6	#	gag	Glu (E)	8	#	aag	Lys (K)	8	#	---	Ter (.)	1
---	Ala (A)	10	#	---	Glu (E)	14	#	---	Lys (K)	8	#	aca	Thr (T)	0
aga	Arg (R)	10	#	gga	Gly (G)	1	#	aug	Met (M)	7	#	acc	Thr (T)	5
agg	Arg (R)	0	#	ggc	Gly (G)	0	#	---	Met (M)	7	#	acg	Thr (T)	0
cga	Arg (R)	0	#	ggg	Gly (G)	0	#	uuc	Phe (F)	7	#	acu	Thr (T)	5
cgc	Arg (R)	0	#	ggu	Gly (G)	4	#	uuu	Phe (F)	1	#	---	Thr (T)	10
cgg	Arg (R)	0	#	---	Gly (G)	5	#	---	Phe (F)	8	#	ugg	Trp (W)	2
cgu	Arg (R)	0	#	cac	His (H)	3	#	cca	Pro (P)	6	#	---	Trp (W)	2
---	Arg (R)	10	#	cau	His (H)	0	#	ccc	Pro (P)	0	#	uac	Tyr (Y)	4
aac	Asn (N)	7	#	---	His (H)	3	#	ccg	Pro (P)	0	#	uau	Tyr (Y)	0
aau	Asn (N)	0	#	aua	Ile (I)	0	#	ccu	Pro (P)	1	#	---	Tyr (Y)	4

6B

---	Asn (N)	7	#	auc	Ile (I)	3	#	---	Pro (P)	7	#	gua	Val (V)	0
gac	Asp (D)	8	#	auu	Ile (I)	4	#	agc	Ser (S)	0	#	guc	Val (V)	3
gau	Asp (D)	4	#	---	Ile (I)	7	#	agu	Ser (S)	0	#	gug	Val (V)	0
---	Asp (D)	12	#	cua	Leu (L)	0	#	uca	Ser (S)	0	#	guu	Val (V)	3
ugc	Cys (C)	0	#	cuc	Leu (L)	0	#	ucc	Ser (S)	5	#	---	Val (V)	6
ugu	Cys (C)	5	#	cug	Leu (L)	0	#	ucg	Ser (S)	0	#	nnn	??? (X)	0
---	Cys (C)	5	#	cuu	Leu (L)	3	#	ucu	Ser (S)	9	#	TOTAL		173
caa	Gln (Q)	10	#	uua	Leu (L)	0	#	---	Ser (S)	14	#			



10

Alignment Report of NLVHVVTU.seqal using Clustal method with PAM250 residue weight table.
Tuesday, May 29, 2001 4:03 PM

CYLSE T L M L D A R E N L K L L D R M N R L S P H S C L			Majority
10 20 30			
1	CYLSRRRLMLDARENKLLDAMNALS PHSCL		IFN-T.OTT.AASEQ.ER
1	CYLSRRRLMLDARENKLLDAMNALS PHSCL		OTP.Y00287.AA
1	CDLPETHSLDNERTIMLLAQMSNIS PHSCL		IFNalphaD.AA
1	CDLPETHSLDNERTIMLLAQMSNIS PHSCL		HVV.AA
1	CYLSRRRLMLDARENKLLDAMNALS PHSCL		NLV.AA
MDRHD F G F P Q E E F D G N Q F Q K A P A I S V L H E L			Majority
40 50 60			
31	QDRKDFGLFQEMVEGDQLCKDQAF PVL YEM		IFN-T.OTT.AASEQ.ER
31	QDRKDFGLFQEMVEGDQLCKDQAF PVL YEM		OTP.Y00287.AA
31	MDRHD F G F P Q E E F D G N Q F Q K A P A I S V L H E L		IFNalphaD.AA
31	MDRHD F G F P Q E E F D G N Q F Q K A P A I S V L H E L		HVV.AA
31	MDRHD F G F P Q E E F D G N Q F Q K A P A I S V L H E L		NLV.AA
I Q Q I F N L F T T K D S S A A W D E D L L D K F C T E L Y			Majority
70 80 90			
61	LOCSFNLFYTEHSSAANDTTLLLEQLCTGLIQ		IFN-T.OTT.AASEQ.ER
61	LOCSFNLFYTEHSSAANDTTLLLEQLCTGLIQ		OTP.Y00287.AA
61	IQQIFNLF TTKDSSA A W D E D L L D K F C T E L Y		IFNalphaD.AA
61	IQQIFNLF TTKDSSA A W D E D L L D K F C T E L Y		HVV.AA
61	IQQIFNLF TTKDSSA A W D E D L L D K F C T E L Y		NLV.AA
Q Q L N D L E A C V M Q E E R V G E T P L M N A D S I L A V			Majority
100 110 120			
91	QQLDHLDTCRGQVMGEEDSELGNMDFIVTV		IFN-T.OTT.AASEQ.ER
91	QQLDHLDTCRGQVMGEEDSELGNMDFIVTV		OTP.Y00287.AA
91	QQLNDLEACVMQEERVGETPLMNADSI L A V		IFNalphaD.AA
91	QQLNDLEACVMQEERVGETPLMNADSI L A V		HVV.AA
91	QQLNDLEACVMQEERVGETPLMNADSI L A V		NLV.AA
K K Y F R R I T L Y L T E K K Y S P C A W E V V R A E I M R			Majority
130 140 150			
121	KKYFQGIYDYLQERKGYSDCAWEIVRVEMMR		IFN-T.OTT.AASEQ.ER
121	KKYFQGIYDYLQERKGYSDCAWEIVRVEMMR		OTP.Y00287.AA
121	KKYFRRITLYLTERKKYSPCAWEVVR AEIMR		IFNalphaD.AA
121	KKYFRRITLYLTERKKYSPCAWEVVR AEIMR		HVV.AA
121	KKYFRRITLYLTERKKYSPCAWEVVR AEIMR		NLV.AA
S L S L S T N L Q E R L T K M G G D L N S P -			Majority
160 170			
151	ALT V S T T L Q K R I T K M G G D L N S P .		IFN-T.OTT.AASEQ.ER
151	ALT V S T T L Q K R I T K M G G D L N S P .		OTP.Y00287.AA
151	SLSLSTNLQERLTKMGGDLN S P .		IFNalphaD.AA
151	SLSLSTNLQERLTKMGGDLN S P .		HVV.AA
151	SLSLSTNLQERLTKMGGDLN S P .		NLV.AA

Decoration 'Decoration #1': Shade (with bright turquoise at 40% fill) residues that match the Consensus exactly.

<110> PEPGEN CORPORATION

<120> Hybrid Interferon/Interferon Tau Proteins, Compositions and
Methods of Use

<150> US 60/311,866

<151> 2001-08-12

<160> 2

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 1 5 10 15

Leu Leu Ala Gln Met Ser Arg Ile Ser Pro Ser Ser Cys Leu Met Asp
 20 25 30

Arg His Asp Phe Gly Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe
 35 40 45

Gln Lys Ala Pro Ala Ile Ser Val Leu His Glu Leu Ile Gln Gln Ile
 50 55 60

Phe Asn Leu Phe Thr Thr Lys Asp Ser Ser Ala Ala Trp Asp Glu Asp
 65 70 75 80

Leu Leu Asp Lys Phe Cys Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu
 85 90 95

Glu Ala Cys Val Met Gln Glu Glu Arg Val Gly Glu Thr Pro Leu Met
 100 105 110

Asn Ala Asp Ser Ile Leu Ala Val Lys Lys Tyr Phe Arg Arg Ile Thr
 115 120 125

Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp Glu Val Val

130

135

140

Arg Ala Glu Ile Met Arg Ser Leu Ser Leu Ser Thr Asn Leu Gln Glu

145

150

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Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro

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170

<210> 2

<211> 519

<212> DNA

<213> Artificial Sequence

<220>

<223> hybrid interferon

<400> 2

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atgtctagaa tctctccatc ctcttgtttg atggatagac acgatttcgg tttcccacaa 120

gaagaatttg acggttaacca attcaaaag gctcctgcta tttctgtttt gcacgagttg 180

attcaacaaa ttttcaactt gttcaccact aaggactctt ctgctgcctg ggacgaagac 240

ttgttggaca agttctgtac tgagctttac caacaattga acgacttgga ggcttgtgtt 300

atgcaagagg agagagtcgg tgagacccca ttgatgaacg ctgattccat cttggctgtc 360

aagaagtact tcagaagaat taccttgtac ttgaccgaaa agaagtactc cccatgtgcc 420

tggaagtcg ttagagccga aatcatgaga tctttgtcct tgtccactaa ctgcaagag 480

agacttacca agatgggtgg agacttgaac tctccataa 519