

(19)
(12)

(KR)
(A)

(51) 。 Int. Cl. ⁷
C07K 19/00

(11)
(43)

2002 - 0009315
2002 02 01

(21) 10 - 2000 - 0043038
(22) 2000 07 26

(71)

3	105 - 706
3	105 1404
3	105 - 702
	101 - 1401
	102 - 303

(72)

3	105 - 706
	102 - 303
3	105 1404
3	105 - 702
	101 - 1401

(74)
:

(54) - 3 , - 3

C3 Tat , ,
Tat - C3 ,

6 Tat - C3

C3 (C3 transferase; " C3 " , " C3")
 Tat - C3 N 6 9 HIV - 1 Tat C3
 (Complement) Rho 가 (immu
 Cdc42 Rac ,
 RhoA . (Caron and Hall, 1998; Massol et al., 1998). Ras Rho
 A E. coli C3 ADP -
 RhoA , C3
 RhoA 가 RhoA가 RhoA
 RhoA 가 RhoA C3 가 C3 가
 RhoA RhoA V14 (fibroblast) (stress fibe
 r) (focal adhesion) (Ridley and Hall, 1992). Rac Rho (endocytosis)
 . Rac Rho (transferrin)
 가 (Lamaz et al., 1996). Rho . Rho가
 Ras (cyclin - dependent - kinase inhibitor;
 CDKI) p21Waf1/Cip1 DNA 가 Rho가
 Ras p21Waf1/Cip1 가 Ras DNA . p21Waf1/Cip1가 R
 as DNA Rho 가 Ras가 Rho p21Waf
 1/Cip1 (Olson et al., 1998).
 (blood - brain barrier)
 HIV Tat (Green, M., and Loew
 enstein). Tat 가 HIV Tat
 120kDa -
 가 가 . Tat 36 -

(heterologous)

(Frankel, A. D., and Pabo).

(transduction domains) (Drosophila) (Antennapedia; Antp)
 (Derossi et al., 1996) HSV HSV VP22
 , Antp 가 4 37
 . Tat 가

Tat - RhoA14V, - RhoA19N (osteoclast) RhoA
 , RhoA (streptolysin) O C3
 가 (Chellaiah et al., 2000). , O
 , Tat - C3 가 C3

C3

가

Tat - C3

RhoA

RhoA

Tat

C3

Tat - C3

HIV - 1 Tat

C3

Tat - C3,

Tat - C3

Tat - C3
 (pET - Tat - C3) C3
 6 (histidine)

, Tat 9
 cDNA

Tat - C3
 (Tat 49 - 57)

Tat - C3
 (metal - chelating affinity chromatography)

t) Tat - C3

Tat - C3

C3

(Western blo

Tat - C3

RhoA

RhoA

C3 HIV1 - Tat (amino acids 49 - 57)
 pTat - C3 , HIV - 1 Tat 9 가

(top strand) : 5' - TAGGAAGAAGCGGAGACAGCGACGAAGAC - 3' ,

(bottom strand) : 5' - TCGAGTCTTCGTCGCTGTCTCCGCTTCTTCC - 3' .

NdeI - XhoI pET15b (Invitrogen, Carlsbad, CA) 6His (open reading frame)
 가 HisTat pHisTat .

(fluorescence - based automated sequencer model 373A; Applied Biosystems, Inc.)

pHisTat , C3 가

Pfu DNA (Clontech) (polymerase chain reaction; PCR) .

(sense primer): 5' - CTCGAGTATTCAAATACTTACCAGGAG - 3' ,

(antisense primer): 5' - GGATCCTTATTTAGGATTGATAGCTGTGCC - 3' .

PCR XhoI - BamHI , pHisTat XhoI - BamHI .
 pTat - C3 , XhoI - BamHI PCR pET15b XhoI - BamHI
 pC3 .

(insert) 가 XhoI - BamHI (Sambro
 ok et al., 1989).

2: Tat - C3

C3 Tat - C3 , C3 Tat - C3 pET - 15b
 His - (tag) 가 Ni2+ - .

pC3 pTat - C3 BL21 (Pharmacia) 100 ug/ml LB 37
 LB 10 O.D₆₀₀ = 1.0 260rpm 37 4
 5 0.1mM IPTG Tat - C3
 6M (protease inhibitors) (

20mg/ml (soybean trypsin inhibitor), 2mg/ml (aprotinin), 5mg/ml (leupept
 in), 100mg/ml PMSF(phenyl methyl sulfonyl fluoride))가 (5mM , 500mM

NaCl, 20mM Tris - HCl, pH 7.9) (cell extract) Ni2+ - IDA 6M 가
 (80mM , 0.5M NaCl, 20mM Tris - HCl, pH 7.9) .
 (eluting buffer; 1M , 0.5M NaCl, 20mM Tris - HCl pH 7.9) PD10 (Amersham)
 Tat - C3 가 .

(bovine serum albumin; BSA) SDS - PAGE

(densitometric analysis)

rad) BSA C3 20% (Bio
 - 80 PBS

C3 tat - C3 35 kDa . Tat - C3 Tat 가 C3
 SDS - PAGE (1)

3: Tat - C3

Tat - C3 , Tat - C3 His -
 - His
 (Macrophage) J774A.1 (cell line) 5mM NaHCO₃, 10% (FBS) 20mM HE
 PES/NaOH(pH 7.4) (100 U/ml, 100U/ml) DMEM(Dulbe
 cco 's Modified Eagle 's Medium) 37 5% CO₂ J774A.1 Tat - C3가
 - EDTA(Gibco BRL) PBS(phosphate buffered saline)

Tat - C3 C3 (2), Tat - C3
 가

4: FITC - (zymosan)

A FITC (label) FBS(5 x 10⁸ /ml가
) (resuspend) , 70

5:

Tat - C3 가 RhoA ADP - RhoA RhoA가
 C3 RhoA FITC - (crystal violet) 가
 FITC -
 2 x 10⁵ 35mm , 5 x 10⁵ FITC - (FITC - conjugated zym
 osan) 30min 37 , PBS PBS 1ml . FITC 490 nm F
 ITC , FITC 520 nm (crystal violet) 가
 3 mM

C3 Tat - C3 RhoA ADP - RhoA
 (3). Tat C3 RhoA ADP - RhoA
 , Tat - C3 Tat - C3 가

6: Tat - C3 RhoA ADP - (ribosylation)

Tat - C3 가 RhoA ADP - RhoA , Tat - C3
 RhoA PAGE SDS - PAGE

Tat - C3 가 RhoA ADP - GST - RhoA
 NAD+ C3 - , RhoA

(Macrophage cells) 37 24 10 - 50 ug/ml C3 tat - C3
 (Cell lysates) SDS - PAGE (nondenaturing) PAGE (14%) . Rho
 - RhoA (in vitro) 30ug/ml C3 (
 Tat - C3), 3 uM NAD, 6 uM GDP, 60 ng/ml GST - RhoA (50 mM HCl, pH 7.5, 2 mM M
 gCl₂, 1 mM DTT, 0.2 mM PMSF) 50ul . ADP - (ribosylation)
 GST - RhoA , 37 1 . RhoA - RhoA
 PAGE SDS - PAGE

ADP - RhoA PAGE (Med. Enzymol; Sehr et al., 19
 98). Tat - C3 RhoA PAGE SDS - PAGE
 . Tat - C3 가 C3 가 RhoA (4).

C3 NAD+ RhoA Rho (5). C3
 , Tat - C3 SDS - PAGE RhoA , PAGE RhoA
 ADP - 가 RhoA (Selkine, et al., 1989).

Tat - C3 C3 (2),
 (3), Tat - C3 RhoA (4).

, Tat - C3 C3 RhoA . Tat -
 C3 at - C3 RhoA RhoA Tat - C3 RhoA T
 RhoA RhoA

C3 가 C3
 Tat

Tat - C3 RhoA

, C3 RhoA

(57)

1.

C3 HIV - 1 Tat 49~57 (Tat 49 - 57)가
 Tat - C3

2.

C3 cDNA 5' HIV - 1 Tat 49~57 (Tat 49 - 57) DNA
 1 Tat - C3 5 가

3.

1 2 Ta
 t - C3

4.

3 , 1A

Tat - C3

5.

3 4 ;

Tat - C3 ;

Tat - C3 가 ; Tat - C3

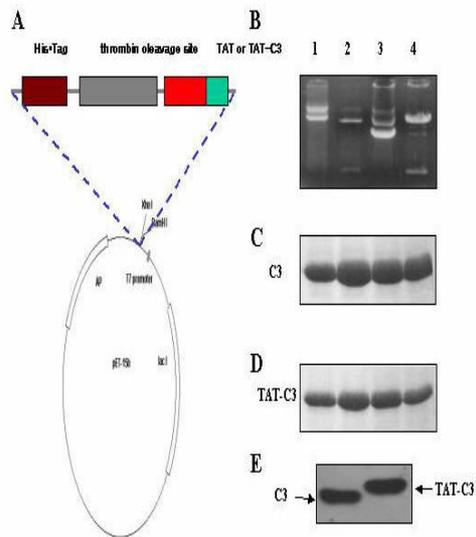
6.

5 , Tat - C3 6M
Tat - C3

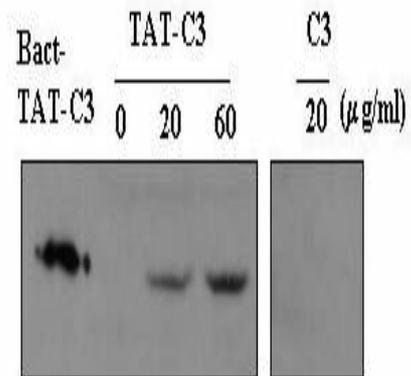
7.

1 TAT - C3

1

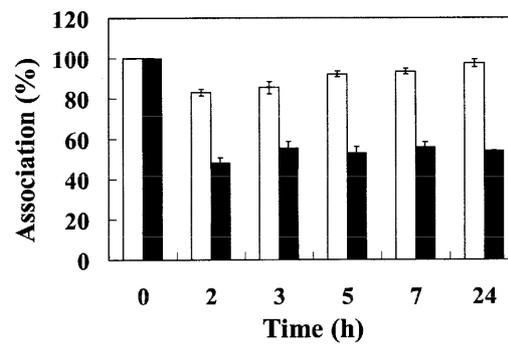


2

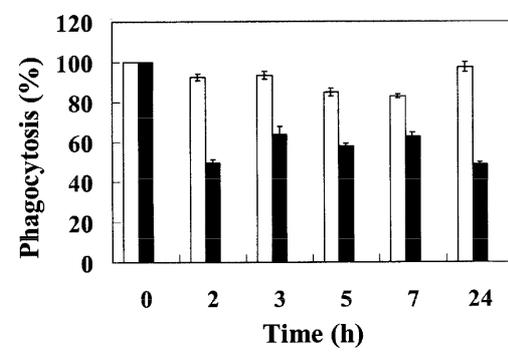


3

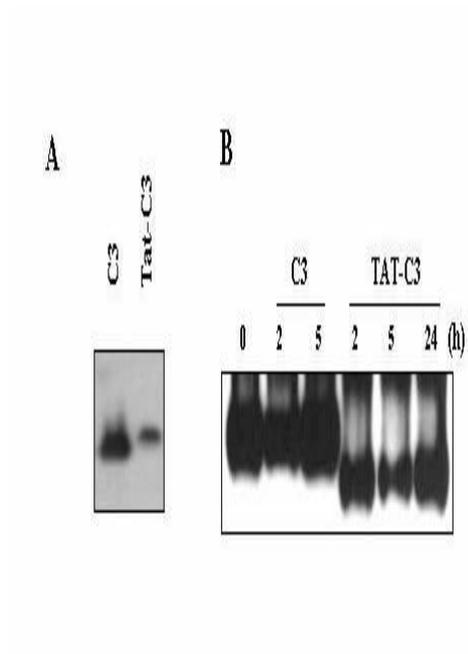
A



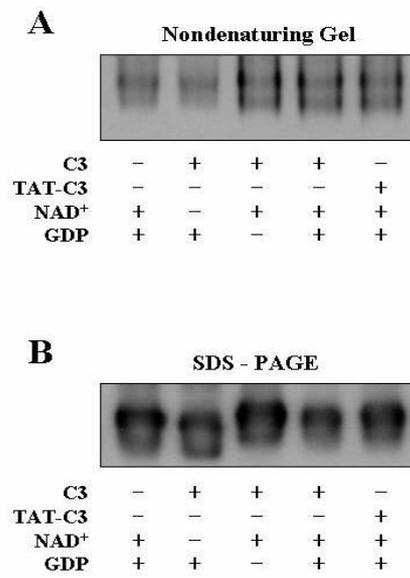
B



4



5



6

Tat-C3 transferase

Tat t agg aag aag cgg aga cag cga cga aga

Xho I

1 c t c g a g a t t c a a a c t t a c c a g g a g t t t a c t a a t a t t g a t c a a g c a a a a g c t t g g g t

61 a a t g c t c a g t a t a a a a g t a t g g a c t a a g c a a a a a g a a g c t a t a g t a t c a t a t

121 a c t a a a a g c g c t a g t g a a t a a t g g a a g c t a a g a c a a a t a a g g a g t t a t c a a t g g a

181 t t t c c t t c a a a t t a a t a a a a c a a g t t g a a c t t t a g a t a a a t c t t t a a t a a a a f g a a g

241 a c c c c t g a a a t a t a t g t t a t t a g a g c g a c g a c c c t g c t a t t a g g a a c a g a a t t

301 c a a a a c a c t c t t c t a a t t c a a t g g t a c a t t a a t a a a c g g c t t t g a a a g g c t a a a

361 g c t a a g t t t t a a a t a a g a t a g a c t t g a a t a t g a t a t a t t a g t a c t t c a t t a a t g a a t

421 g t t t c a a t t g c a g g a a g a c c a a t t a t t a c a a a t t t a a g t a g c a a a a g g c t c a a a g

481 g c a g g a t a t a t g a c c t a t t a g t g t t t t g c a g g a c a a c t t g a a a t g t t g c t t c t a g a

541 c a t a g t a c t t a t c a t a t a g a c g a t a t g a g a t t g t t c t c t g a t g g t a a a c a a t a a t a a t t

601 a c a g c a a c a a t g a t g g c a c a g e t a t c a a t c e t a a t a a g a t a c

BamHI

<110> CHOI, SOO YOUNG PARK, JAE-BONG PARK, JIN-SEO WON,
MOO-HO
LEE, JAE-YONG<120> Cell-transducing HIV-1 Tat-C3 transferase fusion protein, express
ion vector of the fusion protein and the analysing method of Rhop rotein using
the fusion protein&l
t;130> wjtj-shk-tatc3<160> 6<170> KopatentIn 1.55<210> 1<211>
29<212> DNA<213> Human immunodeficiency virus type 1<400> ltaggaagaag cg
gagacagc gacgaagac 29<210> 2<211>
31<212> D
NA<213> Human immunodeficiency virus type 1<400> 2tcgagtcttc gtcgctgtct ccgcttcttc
c
31<210> 3<211> 27<212> DNA<213> Clostridium botulinum<400>
3ctcgagtatt caaatactta ccaggag 27<210>
4<211> 30<
212> DNA<213> Clostridium botulinum<400> 4ggatccttat ttaggattga tagctgtgcc
30<210> 5<211> 673<212> DNA<213> Artificial Sequence<220>&
lt;223> recombinant polynucleotide fused polynucleotide coding HIV-1 Tatp
rotein and polynu
cleotide coding Human C3 transferase<400> 5taggaagaag cggagacagc gacgaagact
cgagtattca aatac
ttacc aggagtttac 32taatattgat caagcaaaag cttggggtaa tgctcagtat aaaaagtatg
gactaagcaa 92atcag
aaaaa gaagctatag tatcatatac taaaagcgct agtgaaataa atggaaagct 152aagacaaaat
aaggagatta tcaatggatt tc
cttcaaat ttaataaaac aagttgaact 212tttagataaa tcttttaata aatgaagac cctgaaaat
attatgttat ttagaggcga
272cgaccctgct tatttaggaa cagaatttca aacactctt cttaattcaa atggtacaat
332taataaaaacg gcttttgaaa aggct
aaagc taagttttta aataaagata gacttgaata 392tggatatatt agtacttcat taatgaatgt
ttctcaattt gcaggaagac caat

tattac 452aaaatttaaa gtagcaaaag gctcaaaggc aggatatatt gaccctatta gtgcttttgc
512aggacaactt gaa
atgttgc ttctagaca tagtacttat catatagacg atatgagatt 572gtcttctgat ggtaaacaaa
taataattac agcaacaatg a
tgggcacag ctatcaatcc 632taaataagga tcc
645<210>
6<211> 9<212> PRT<213> Human immunodeficiency virus type 1<400>
6Arg Lys Lys Arg Arg Gln Arg Arg Arg 1 5