

(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)

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3	105 - 702
	101 - 1401
	102 - 303

(72)

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3	105 - 702
	101 - 1401

(74)
:

(54) - 3 , - 3

C3 Tat , ,
Tat - C3 ,

C3 N 6 9 HIV - 1 Tat C3

Tat - C3 RhoA Tat - C3 RhoA Tat - C3 RhoA Tat - C3

4

Tat, C3 , RhoA,

1 C3 Tat - C3 pET15b

pETC3 pET - Tat C3 (A). pET - C3 Tat - C3 가
 1, 3: intact plasmids, 2, 4: pET, C3 Tat - C3 (B). C3 Tat - C3 His -
 SDS - PAGE (C, D) - C3 (E)

2 Tat - C3 J774A.1

J774A.1 (5×10^5) 20 - 60ug/ml Tat - C3 C3 37 1
 SDS - PAGE - His

3 C3 Tat - C3

(association) 37 C3 () Tat - C3 () 50 ug/ml
 (fluorescence spectrophotometer) 3mM

4 J774A.1 RhoA ADP -

C3(50 ug/ml) tat - C3(50 ug/ml) 37 24
 SDS - PAGE(14%)(A) PAGE(14%)(B) Rho

- RhoA

5 (In vitro) Tat - C3 RhoA ADP -

50 ul (50mM HCl, pH 7.5, 2 mM MgCl₂, 1 mM DTT an
 d 0.2 mM PMSF) 30 ug/ml C3 Tat - C3, 3 uM NAD⁺, 6 uM GDP, 60 ng/ml GST - RhoA
 . GST - RhoA ADP - , 37 1 . Rho
 PAGE(A) SDS - PAGE(B) , - RhoA

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)
 enstein). HIV Tat 가 (Green, M., and Loew
 Tat 120kDa - HIV Tat
 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). , Tat - C3 가 C3 O

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
 C3 RhoA ADP - RhoA RhoA가
 FITC - FITC -
 (crystal violet) 가

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

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

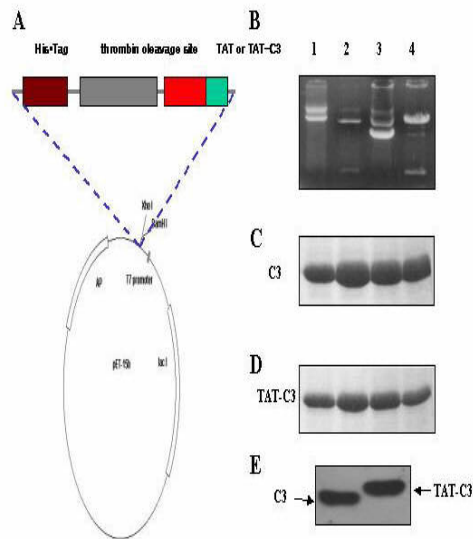
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5 , Tat - C3 6M
Tat - C3

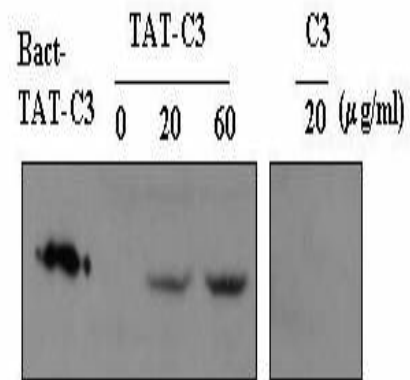
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1 TAT - C3

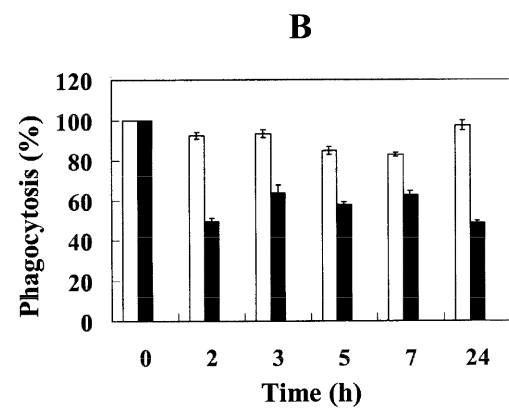
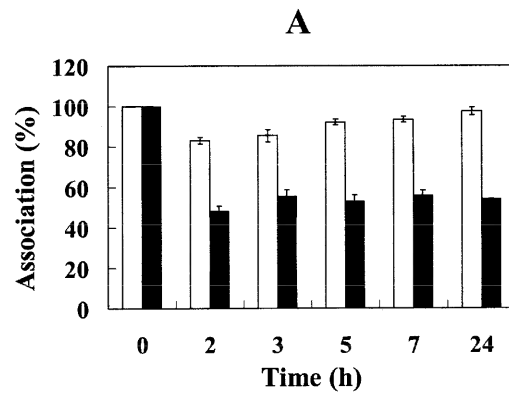
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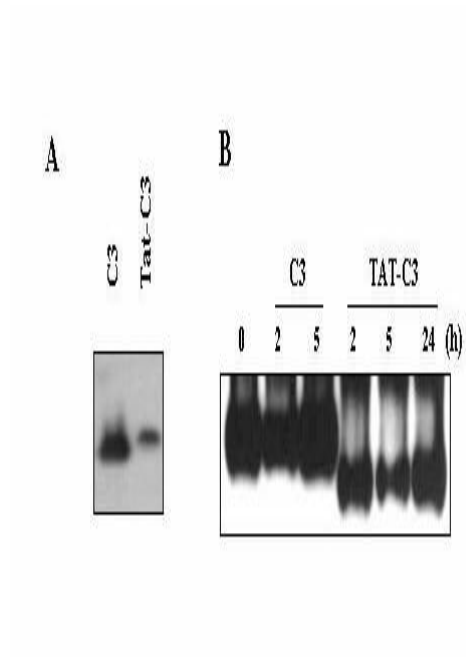
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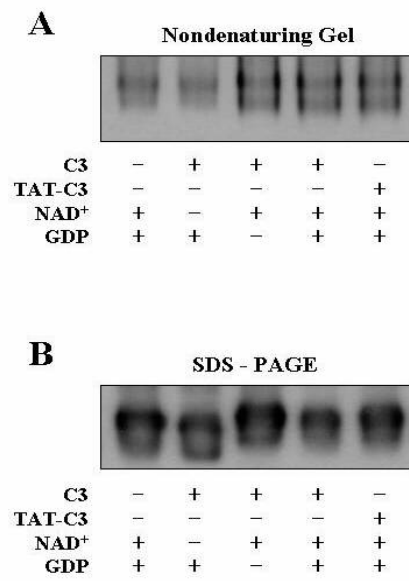
3



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

BamHI

<110> CHOI, SOO YOUNG PARK, JAE-BONG PARK, JIN-SEO WON,
MOO-HO
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