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(12)

(KR)
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A61K 48/00

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(11)
(24)

2004 11 10
10-0456681
2004 11 02

(21) 10-2002-0028505
(22) 2002 05 22

(65)
(43)

10-2003-0090897
2003 12 01

(73)

223-23

3 187-114

(72)

가 2 80-145

106 204

36

212-1005

1403 1103

494-86

4

412-601

(74)

:

(54)

DNA

DNA

1 DNA
 2.0 -0.5kb, 3 0.5-0.1kb, 4 0.1kb DNA(Intact, 10kb), DNA
 2 2-0.5kb DNA(CIA02)가 가 5 (Batch)
 3
 4 가 (CIA04), 2
 5 (CIA05) THP-1 TNF-
 6a (CIA05)
 6b (CIA05)
 7 DNA(CIA02) (CIA05) 가
 8 (CIA07) 가
 9 (CIA07)
 10 (CIA07) Raw
 NF-kB가 promoter 가

DNA
 1960 , , 3 1973
 가
 1) ;
 2: 492-494) ;
 2) ;
 - (Active specific immuization)
 가 X mitomycin-C
 가 neuramindase 가 Con-A
 가 , dendritic (DC) (Sprinzl et al , *Cancer Treat Rev* . 2001 Au
 g;27(4):247-55) DNA (Pantuck et al, *Int J Urol* . 2001 Jul;8(7):S1-4)가
 가
 가
 Corynebacterium , Picibanil(OK-432)

1968 Bush Fehleison 1891 Coley Coley streptococci

- BCG() Old Mathe BCG BCG BCG

BCG : 1960 Morton BCG tuberculin 가 .)

1970 BCG

- Unmethylated CpG DNA DNA CpG CpG CpG 가

가 B IgM 가 c-myc , -IgM myn, blc2, CpG bcl-XL mRNA 가 B

가 B CpG IL-6 IL-12 CPG

pG . BCG , CpG 가 가 가 BCG C

DNA 가 2.0-0.1kb , 2.0-0.5kb

DNA 3 - DNA (dose) 가 , T 1

500:1 - 1:500 가 E.coli EG0021(KCCM-10374) 가 CIA02, CIA05

CIA07 DNA 가 T (sepsis)

가

361-221 (*E.coli* EG0021) KCCM 10374 2002 5 2

가 DNA DNA(CIA02) *E. coli* EG0021 DNA
 DNA (fragmentation) CIA02 CIA05 CIA02 CIA07
 CIA02

1:

가

(*E.coli* EG0021)

가

Balb/C

50 5 50 0.9% 4m
 1ml DNase 1 2ul 37 incubator 1 . D
 Nase 1 10mg/ml RNase 50ul 37 incubator 1 20mg
 /ml Proteinase K 100ul 37 TNF-a 가 LPS GM-C
 SF (1) (3
 (ladder)가 5 -1
 EG0021

[1]

TNF-a

No.	TNF-a (pg/1ul)	No.	TNF-a (pg/1ul)	No.	TNF-a (pg/1ul)	No.	TNF-a (pg/1ul)	No.	TNF-a (pg/1ul)
EG0001	(>100)	EG0011	39	EG0021	0.1	EG0031	21	EG0041	65
EG0002	12	EG0012	64	EG0022	(>100)	EG0032	1.2	EG0042	54
EG0003	72	EG0013	8.8	EG0023	(>100)	EG0033	(>100)	EG0043	(>100)
EG0004	85	EG0014	9	EG0024	(>100)	EG0034	(>100)	EG0044	(>100)
EG0005	25	EG0015	70	EG0025	53	EG0035	7	EG0045	17
EG0006	35	EG0016	(>100)	EG0026	12	EG0036	87	EG0046	2.1
EG0007	71	EG0017	6	EG0027	4	EG0037	0.7	EG0047	3.5
EG0008	28	EG0018	11	EG0028	76	EG0038	39	EG0048	(>100)
EG0009	2	EG0019	0.3	EG0029	92	EG0039	37	EG0049	(>100)
EG0010	13	EG0020	80	EG0030	(>100)	EG0040	91	EG0050	(>100)

2: DNA

E. coli EG-0021 37 , TSB(Tryptic soy broth; Difco) 30g/l 10
 10L 8,000G 150g 300 ml TE(10mM Tris, pH 8.0, 25 mM EDTA)
 150g 750mL (10mM Tris (pH 8.0) , 25 mM EDT
 A. 100 g/ mL Lysozyme) 37 1
 proteinase K (Sigma) 100g/ml 가 50 12

/ / (25:24:1) 1:1 3 .

DNA UV 260nm, 280nm

가 DNA (ug/ml) = OD260nm X X 50
 가 DNA (ug/ml) = OD260nm X X 40
 OD260nm / OD280nm = 1.7 ~ 1.8

TE 0.5mg/ml
 - Sonics 500watt sonication VCX500 630-0220(tip diameter :1/2'(13m
 m) 20ml

- DNA size (1). DNA 20
 ,000J act, 10kb), 2.0~0.5kb, 0.5~0.1kb, 0.1kb DNA(Int
 - DNA DNA
 (2). HEL (Sigma) 50ug DNA50ug (adjuvant) ICR
 (, 4 , 20g) 0.1ml 2 7
 가 HEL 가 가 ELISA (2a).
 2.0 ~ 0.5kb 가 가 가 1kb
 DNA가 가 ELISA DNA (2b, 2c).
 3: DNA 1kb DNA 20,000J 7
 (endotoxin)
 DNA DNA 4 , 12 3
 Triton X-114 (Sigma) 0.5%가 4 , 4 37 5
 DNA / / (25:24:1) 1:1 DNA (pyrogen)
 DNA Limulus Amebocyte Lysate (LAL) (BioWhittaker QCL - 1000)

1 DNA(CIA02)

[2] DNA(CIA02)

	DNA	DNA (/15ml)	DNA	(DNA 1mg/ml)	
1	3mg/ml	45mg	16.2mg	1.77	< 1ng 36%
2			20.25mg	1.66	< 1ng 45%
3			18.9mg	1.71	< 1ng 42%

- GC/MSD (gas chromatography/mass selected detector)
 HP - 5890A/HP - 5870B Column 50m.ultra - 1, SIM (Selected Ion Monitoring)
 (2).

[3]

ng/ul	-	0.813	-	-
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- Brad-Ford DNA mg , 99%
 4: (CIA 04)

DNA 4,000g 1.5
 가 4,000g 가 4,000g
 1g 7.5ml (90%)

: (Petroleum) = 2:5:8 가 .
 , 25 , 3000rpm (1,200g) 20 12 가
 , 25 , 3000rpm (1,200g) 20
 가

Biological Lab.) SDS-PAGE KDO (2-Keto-3-deoxyoctonate) (Lsit
 (3).

5
 5:
 A
 3mg/ml 0.2N NaOH 1:1 60 10
 140 1/5 1N pH 7.0
 - 0.2N NaOH
 - pH
 - KDO SDS-PAGE
 - A가
 가 (4).

(Inflammatory)

THP-1(Acute monocytic leukemia)
 가 TNF-
 1ug 5pg TNF-
 1ug DNA 0.5 pg TNF- 50 가
 TNF- 100fg
 (5).

A. 350 g 5
 가
 2 1 1 5ml 5
 B. 5 5 가
 2 1 7

가 (6a).

3 1kg
 0.2ug/1ml 3
 1.5kg 250 30 가
 0.1 16
 가 가
 60-90mm

가 15 37
 가 (差) 3 1 3
 3 가 1.3 , 2.5
 3 4

[4]

횟수	번호	투여 전 (3회 측정)			투여 후 (단위 : 시간, 30분간격)						상승 체온	상승 체온 누적 합계	결과	기준
		1	2	3	0.5	1	1.5	2	2.5	3				
1회	1번 토끼	39.1	39.2	39.2	39.4	39.3	39.2	39.2	39.1	39.1	0.2	0.8	통과	1.3°C 이하
	2번 토끼	39	39.1	39.3	39	39.2	39.5	39.2	39.1	39.3	0.4			
	3번 토끼	39.4	39.2	39.2	39.3	39.5	39.3	39.5	39.3	39.4	0.2			
2회	1번 토끼	39	39.3	39.1	39.4	39.2	39.3	39.1	39.2	39	0.4	1.7	통과	3.0°C 이하
	2번 토끼	39.4	39.2	39.2	39.1	39.1	39.3	39.1	39.2	39.2	0.3			
	3번 토끼	39.3	39.3	39.2	39.4	39.4	39.4	39.4	39.4	39.3	0.2			
3회	1번 토끼	39.2	39.2	39.1	39.2	39.2	39	39.2	39.1	39.1	0.2	2.5	통과	5.0°C 이하
	2번 토끼	39.1	39.5	39	39	39.1	39.2	39.1	39.3	39.2	0.4			
	3번 토끼	39.2	39.3	39.2	39.3	39.2	39.3	39.2	39.4	39.3	0.2			

6: DNA(CIA02), (CIA05) 가
DNA(CIA02) (CIA05)
가 , DNA(CIA02), (CIA05)
2가 (MPBH,
SPB) CIA02 CIA05 CIA02, CIA05,
HEL (Sigma) 50ug ICR (, 4 , 20g) 0.1ml 2
(7a). 가 HEL ELISA
LPS(CIA04) 0.5, 1ug CIA05 0.5ug, CIA02 50ug, CIA05 1ug, CI
A02 50ug CIA05 0.5ug, CIA02 50ug, CIA05 1ug, CIA02 50ug
LPS(CIA04)가 가 가
(7a). ,
(7b). , IgG2a 가
CIA05
, 0.5ug 1ug 1ug ELISA 1ug (7b, 7c).
(8a).
CIA07 가 HEL (Sigma L-6876) 50ug
ICR (, 4 , 20g) 0.1ml 2 7
가 HEL ELISA
CIA07 가 CFA(complete Freund`s adjuvant)
Alum (aluminium hydroxide gel) (8a). , isotype swi
tching CIA07 CFA, Alum IgG1 가
Th(T helper) 2 가 , CIA07 IgG1 IgG2a 가
Th(T helper) 1 type 가 (8b, 8c).
CIA02 25ug, 50ug CIA05 1ug 가
가 CIA02 50ug, CIA05 1ug
(whole blood) CIA 가
RPMI 1640 (2mM L- glutamine, 1mM Sodium pyruvate, 80ug/ml gentamycin) 1:1
1ml CIA07 20ul (CIA02 50ug+CIA05 1ug 500ng, 100ng) HBSS 20ul 37
, 5% CO₂ 24 IFN- (Ramp;D , 210- TA
-010), IL-12 p70(Ramp;D , 219-IL-005) ELISA
9
- CIA02 CIA05
CIA02 50ug, CIA05 1ug 가
Luciferase assay
Raw 12 5x10⁴ (1ml DMEM / 10% FBS) 24 37 , C
O₂ incubator . IL-12 Luciferase Reporter (0.2 mg/), PRL-null (20ng/
) , Serum free DMEM (50 ml/) Fugene 6(1.5ml/ , Roche Cat. No. 1 814
443) 5 10 Raw 52
ml/ , 37 , CO₂ incubator 24 CIA07(CIA02 20mg +CIA0

5 400ng) Raw omega Cat.no. E1500) 12 Luciferase 37 , CO₂ incubator Luminometer . Luciferase assay (Pr Luciferase activity

CIA07 IL-8 IL-12 promoter luciferase , IL-8 IL-12 promote r NF-kB binding site가 , RAW264.7 CIA07 NF-kB가 prom oter 가 (10).

CIA

- CIA07 51 Cr-release 5~8 , C3H/HeN RPMI-1640(10mM HEPES, 100units/ml penicillin, 100µg/ml streptomycin , 300µg/ml glutamine; Gibco Laboratories, Grand Island, NY) 56 30 가 10% fetal bovine serum(Gibco Laboratories, Grand Island, NY) 가 . LAK Sarcoma 180 MBT-2

가 phosphate buffered saline 가 100 micron 가

1 37 0.84% 5 37 , 5% CO₂ 1

2 5x10⁶ 5x10⁶ /ml

10⁶ 300g 3 가 0.2-0.3ml . 100 Ci Na₂⁵¹CrO₄ (1ml Ci/ml, trypan blue 3

NEZ 030S, NEN, USA) 가 37 1 5x10⁴ /ml 5x10³ 가 가 0.1ml 가 . 37 96 5x10³ 가 가 100:1 0.1ml 가 . 500 , 5% CO₂ 4 3 4 0.1ml gamma counter(Packard, USA) 5% triron X-100(Sigma, USA) 0.1ml 가

MR : 5% Triton X-100 (cpm)

[Cytotoxicity](%) = (ER-SR / MR-SR) X 100

ER : (cpm)

SR : (cpm)

MR : 5% Triton X-100 (cpm)

1.5 , LAK , MBT-2 8 가 BCG 가 BCG CIA07 가 5

[5]

Sarcoma 180				
	0	3	7	15
	100	100	100	100
BCG	92 ± 4	110 ± 2	632 ± 13	189 ± 4
CIA07	94 ± 7	154 ± 3	802 ± 10	109 ± 7
MBT-2				
	100	100	100	100
BCG	103 ± 3	96 ± 7	402 ± 11	98 ± 3
CIA07	97 ± 4	121 ± 9	513 ± 13	109 ± 6

BCG , 가 (CIA02, CIA05) , 가 (CIA07) DNA 가 CpG ,
 , (CIA07) 가

(57)

1. a) 2.0-0.5kb DNA ;
 b) 3 -

2.

3.

4.

1 DNA , 500:1 - 1:500

5.

1 DNA ,

6.

1 ,

7.

1 ,

8.

1 , T 1

9.

1 ,

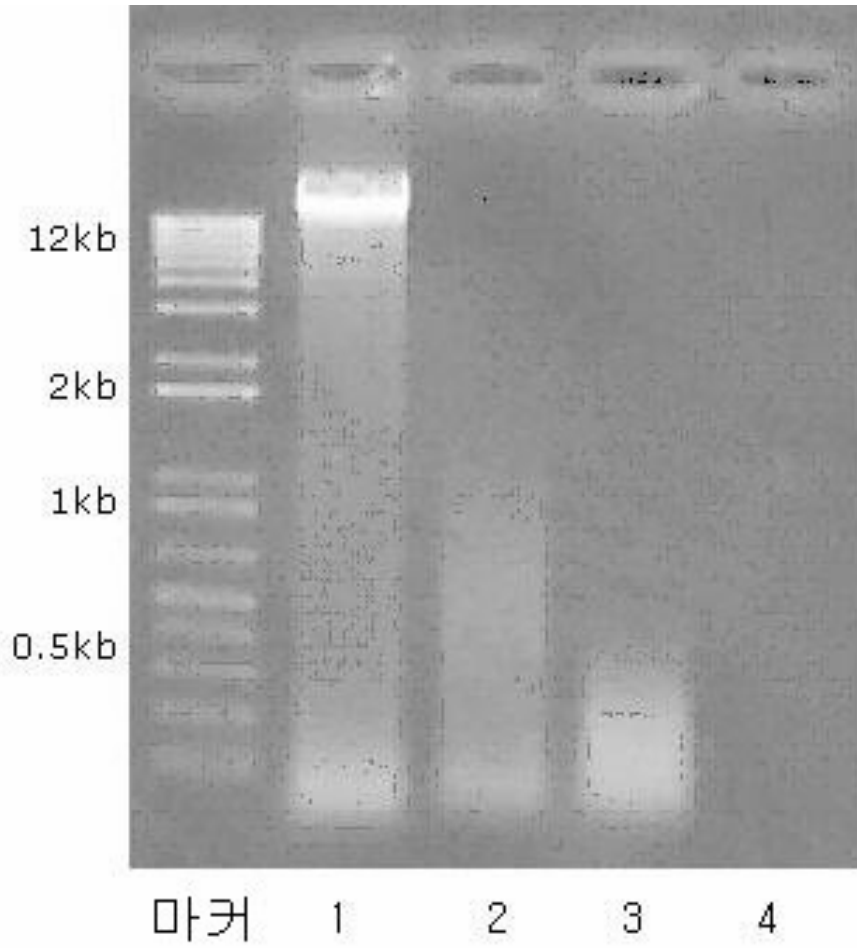
10.

1 9 ,

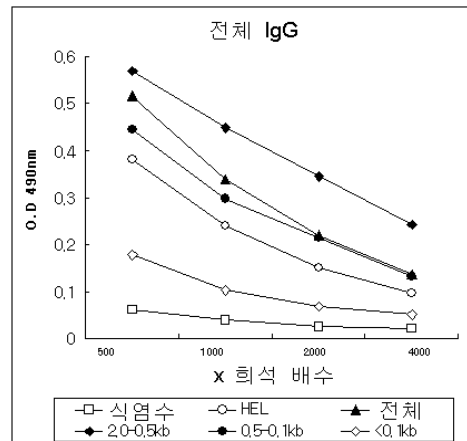
11.

10 , E.coli EG0021(KCCM-10374)

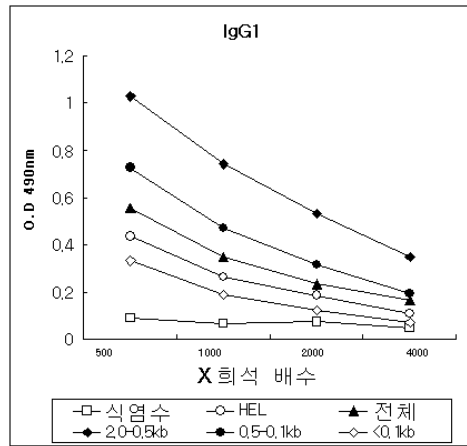
1



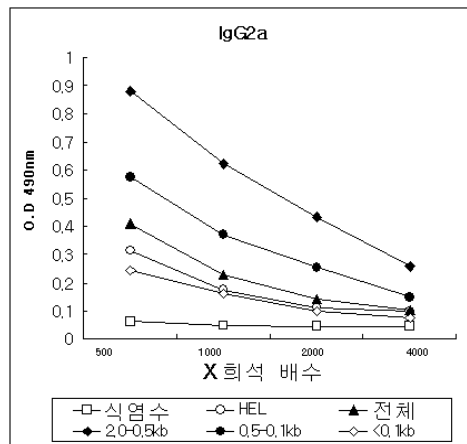
2a



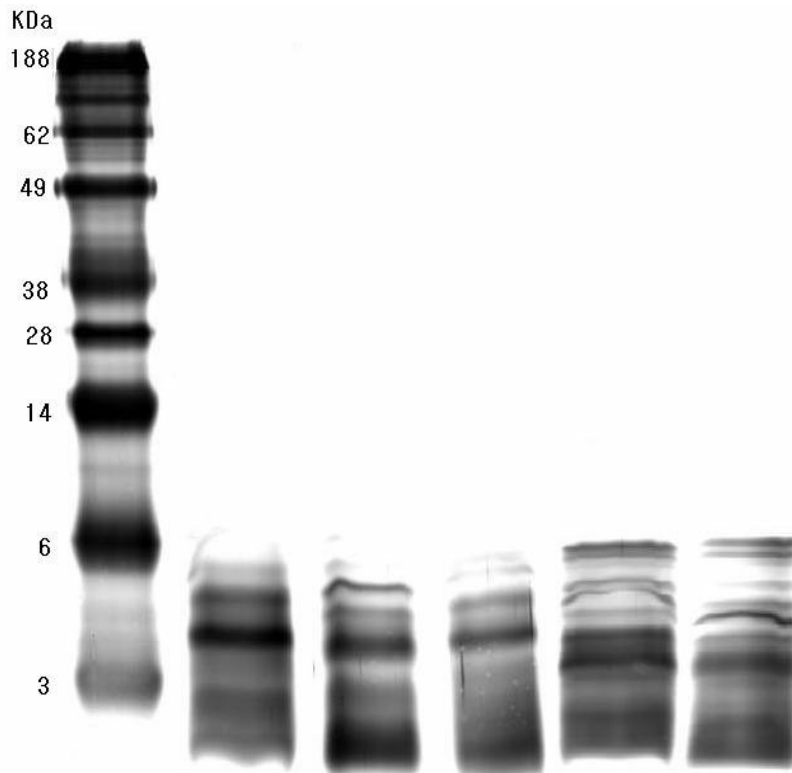
2b

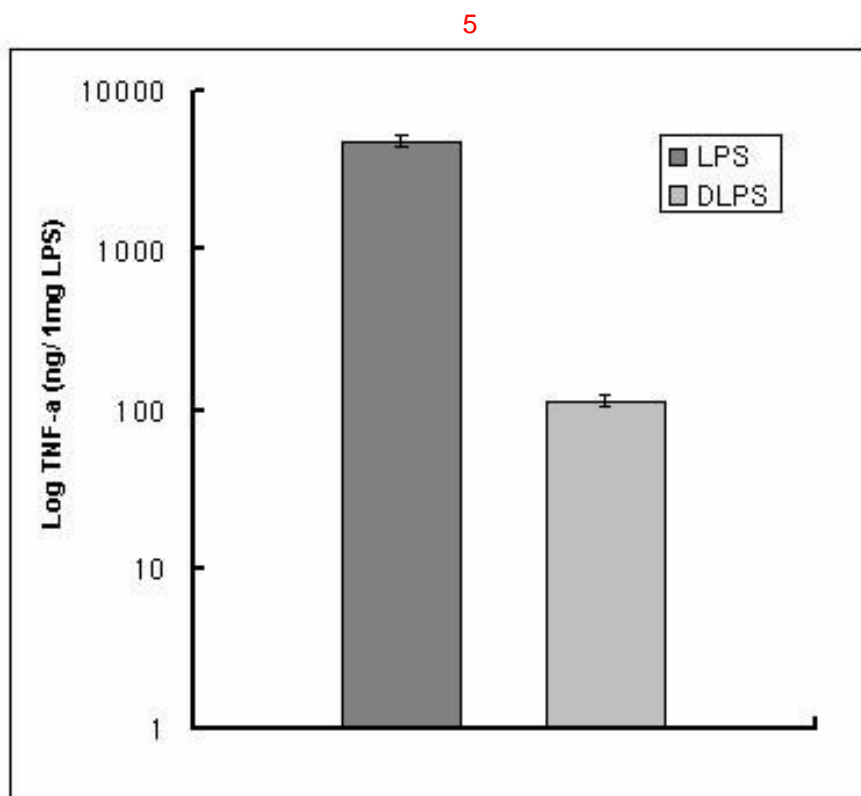
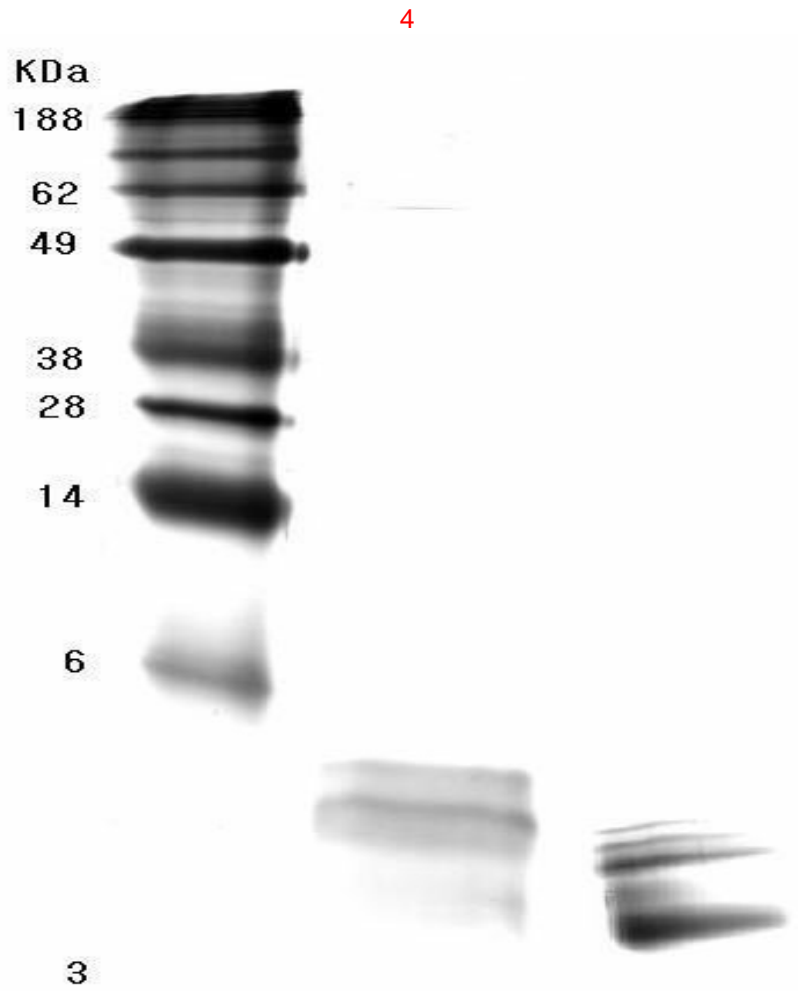


2c

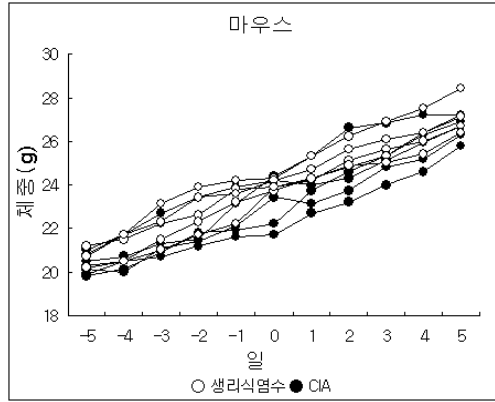


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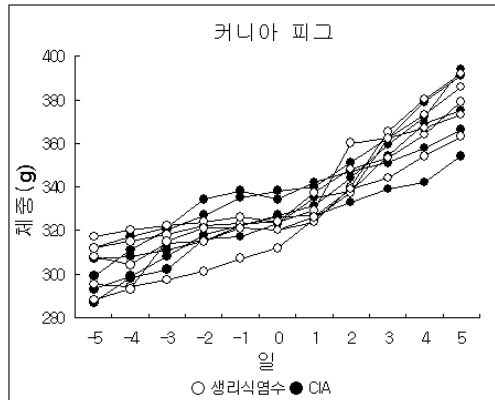




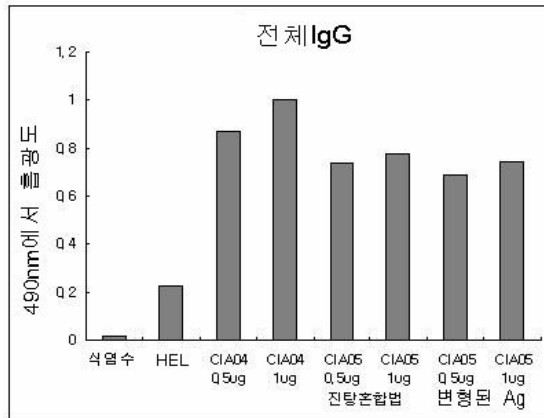
6a



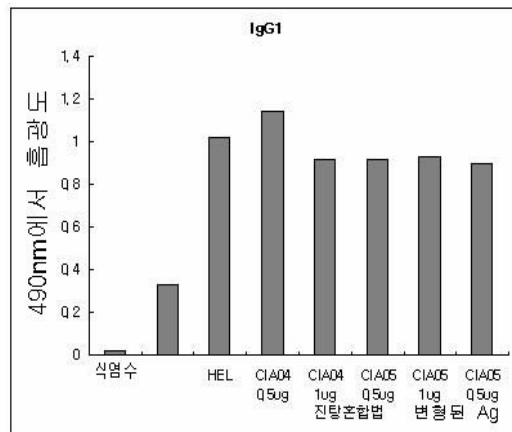
6b



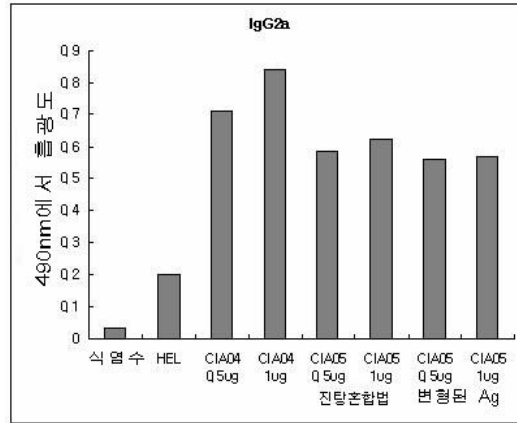
7a



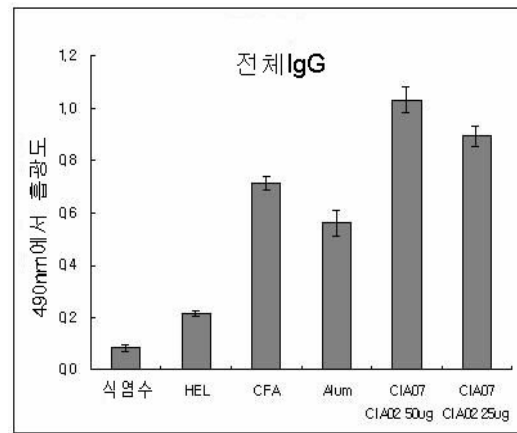
7b



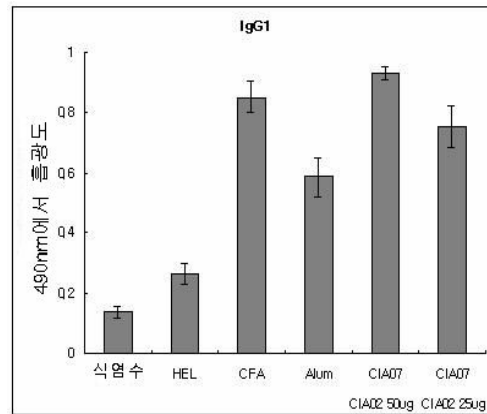
7c



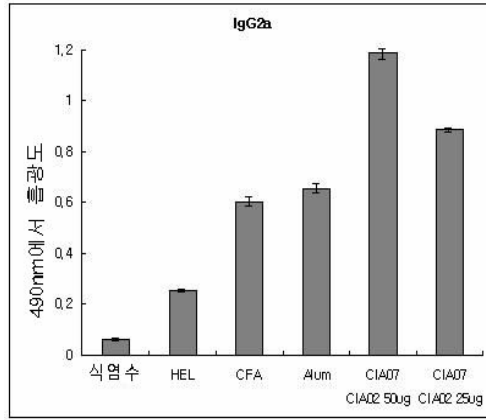
8a



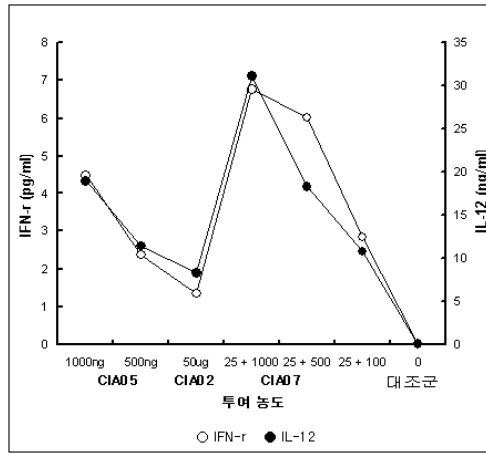
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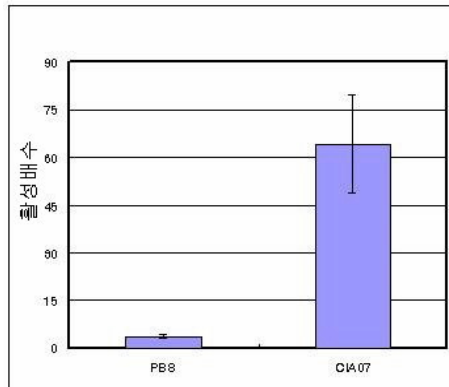
8c



9



10a



10b

