

(19)
(12)(KR)
(A)(51) 。 Int. Cl. ⁷
A61K 38/31(11)
(43)2001 - 0083966
2001 09 06(21) 10 - 2000 - 0006175
(22) 2000 02 10(71) 1 870 542 - 1104
3 611 214 2001(72) 1 870 542 - 1104
3 611 214 2001
103 606
87 303 1105

(74)

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(54)

(MMP - 2)

1a MT1 - MMP proMMP - 2가 MMP - 2

1b APMA proMMP - 2가 MMP - 2

2 PMA proMMP - 2가 MMP - 2

3a proMMP - 2 MMP - 2

3b 3a MMP - 2

4 MT1 - MMP MMP - 2

5a FLAG FLAG

5b FLAG MMP - 2

s) (endostatin) (matrix metalloproteinases, MMP

가 (embryonic development),
(tumor metastasis),
(: Cawston, Br. Med. Bull., 51:385 - 401, 1995).
MMP - 1(matrix metalloproteinase - 1) MMP - 8(matrix metalloproteinase - 8)
(collagenase) , MMP - 2 MMP - 9 (gelatinase) , MMP - 3 MMP - 10
(stromelysin) , MMP - 14 MMP(membrane type MMP)
MMP N - (pro - dom
ain) , N - MMP
(: Stetler - Stevenson et al., J. Biol. C
hem., 265:13933 - 13938, 1990). MMP MMP - 2 (basement membrane) typ
e (type collagen) , MMP - 2 proMMP - 2
MMP - 2 MMP(soluble MMP)
1 (membrane type - 1 MMP, MT1 - MMP)
(: Sato et al., Nature, 370:61 - 65, 1994)

, (murine hemangioendothelioma) 가
 20kDa 가 , (xenograft)
 (: O'Reilly M.S. et al., Cell, 86:353 - 364, 1994; Boehm T. et al., Nature, 390:404 - 407, 1997),
 (: Boehm T, et al., Nature, 390:404 - 407, 1997). ,
 (vascular endothelial growth factor, VEGF) bFGF(basic fibroblast growth factor)
 (apoptosis)

(in vitro) (in vivo)

MMP - 2 proMMP - 2(promatrix metalloproteinase - 2) MMP - 2 MT1 - MMP
 P , MMP - 2

, cDNA (polymerase chain reaction, PCR) , PCR pPFLAG - CMV - 1
 , FLAG
 20kDa PAGE , FLAG 가
 , MMP - 2 proMMP - 2 MMP -
 2 sMT1 - MMP Sf9 , MMP
 - 2 proMMP - 2 MT1 - MMP

proMMP - 2 , PMA(phorbol 12 - myristate 13 - acetate)
 - 2 . , FLAG proMMP - 2 MMP
 (immuno - depleted) , proMMP - 2 MMP
 pro - MMP - 2 , MT1 - MMP
 proMMP - 2 (pro - domain) proMMP - 2 MMP - 2 , APMA(p - aminop

henylmercuric acetate) proMMP - 2 MMP - 2 가 ,
 MT1 - MMP APMA proMMP - 2 ,
 MT1 - MMP APMA proMMP - 2 ,
 MMP - 2 MMP - 2 , MT1 - MMP
 proMMP - 2 MMP - 2 ,
 sMT1 - MMP proMMP - 2 MMP - 2 ,
 proMMP - 2 MMP - 2 , MT1 - MMP

MT1 - MMP proMMP - 2 MMP
 , MMP - 2 .

가
 가

1:

(human umbilical vein endothelial cells, HUVEC)
 20%(w/v) FBS, 100units/M ℓ , 100 μ g/M ℓ , 3ng/M ℓ bFGF(basic fib
 roblast growth factor, Upstate Biotechnology, U.S.A.) 5units/M ℓ M19 (Life Techno
 logies, U.S.A.)가 75cm² , 37 5% CO₂ .
 (human fibrosarcoma cell) HT1080 (ATCC CCL121)
 (human embryokidney cell) HEK293 () 10% FBS,
 100units/M ℓ , 100 μ g/M ℓ (DMEM, Life Technolo
 gies, U.S.A.) .

2:

C 183 cDNA
 PCR : 1) , 3' cDNA 5' GCGAAGCTTCATACTCATCAGGACTT
 T - CAGC(: 1) , 3' GGGGTCGACCTATTTGGAGAAAGAGGTCATG(: 2)
 PCR , PCR Hind /Sa1I , N
 FLAG(DYKDDDDK) (epitope) (linker) pFLAG - CMV - 1
 (KODAK, U.S.A.) pFLAG - CMV - 1 - endostatin 1
 HEK293 pFLAG - CMV - endostatin pcDNA3.1(Clontech, U.S.A.) , G
 418(0.6mg/M ℓ) ,
 (: Sasaki T. et al.,EMBO J., 17:4249 - 4256(1998)).
 , 0.1 μ g/M ℓ 200 μ l FLAG (KODAK, U.S.A.)가 100 μ l
 FLAG 가 A (Upstate Biotechnology, U.S.A.) 100 μ l 4 24
 , 4000rpm 2 10 μ l 15% SDS - PAGE .
 FLAG DYKDDDDK
 HT1080(ATCC CCL121) , MMP - 2

3:

MMP - 2, MT1 - MMP가 proMMP - 2 (pro - domain)
 (: Sato et al., J. Biochem., 119:209 - 215, 1996), proMMP - 2
 (transmembrane - deleted) sMT1 - MMP(soluble membrane type - 1 MMP)

Sf9(ATCC CRL1711) (: Jo et al., J Biochem. Mo
 l. Biol., 32:60 - 66, 1999).

4: proMMP - 2 MMP - 2

proMMP - 2 MMP - 2, 20mM Tris - HCl(pH 7.5), 150mM NaCl, 5mM CaCl₂, 100 μ M ZnCl₂ 0.025% Brij 35 MMP 40μℓ 0, 0.31, 0.63, 1.3, 2.5, 5.0, 10 20μg/ℓ 10ng proMMP - 2 12ng sMT1 - MMP 가 37 3 . , APMA proMMP - 2 proMMP - 2 가 (: Okada et al., Eur. J. Biochem., 194:721 - 730, 1990), proMMP - 2 , 12ng sMT1 - MMP 1mM APMA(Sigma Chemical Co., U.S.A.) 가 37 30 . 0.1% 9% SDS - PAGE , 2.5% Triton X - 100 SDS 50mM Tris - HCl(pH 7.5), 150mM NaCl 10mM CaCl₂ 24 , 0.25% R250 .

1a MT1 - MMP proMMP - 2가 MMP - 2 . 1a , 1 proMMP - 2(68kDa) , 2 9 12ng sMT1 - MMP가 proMMP - 2 proMMP - 2 1a 2 , proMMP - 2 (68kDa) MT1 - MMP , MMP - 2가 64kDa (intermediate form) (64kDa) , 3 9 .

1b APMA proMMP - 2 MMP - 2 proMMP - 2(68kDa) , 2 9 1mM APMA가 proMMP - 2 proMMP - 2(62kDa) 1b 2 , 3 9 . MP - 2(62kDa) - 2(62kDa) .

, sMT1 - MMP APMA proMMP - 2 MMP - 2 ,

5:

4 MT1 - MMP APMA proMMP - 2 MMP - 2 , proMMP - 2 MMP - 2 .

1 60ng/ℓ PMA 0.1, 1, 3, 5 10mg/ℓ

37 24 , 10 μ l ,
 60ng/ml PMA (Alexis, U.S.A.)
 (immuno - depleted) 37 24 , 10 μ l ,
 (immunoblotting) ,
 FLAG , MMP - 2 pr
 oMMP - 2 MMP - 2 .

2 PMA (phorbol 12 - myristate 13 - acetate) proMMP - 2가 MMP - 2
 . 2 , 1 , 2
 PMA , 3 8 2
 . 4 , proMMP - 2 (68kDa) PMA MMP - 2 (62kDa)
 , MMP - 2 (62kDa) .

3a proMMP - 2 MMP - 2
 , 3b 3a proMMP - 2 MMP - 2
 MMP - 2 , 2 PMA , 3 PMA
 , 4 , 5 4
 A , PMA가
 1, 3 5 proMMP - 2 (68kDa)가 MMP - 2 (62kDa)
 2 4 proMMP - 2가 MMP - 2 .

proMMP - 2 MMP - 2가 , PMA proMMP - 2가 MMP - 2
 , , proMMP - 2 MMP - 2 ,

6: MT1 - MMP MMP - 2
 4 5 , proMMP - 2가 MMP - 2
 , MMP - 2
 , 3 proMMP - 2 1mM APMA 37 30 MMP - 2
 , Mca - Pro - Leu - Gly - Leu - Dpa - Ala - Arg - NH₂ (Bachem, U.S.A.)
 (: Yamamoto et al., J. Med. Chem., 41:1209 - 1217, 1998).
 , 20ng MMP - 2 20ng MT1 - MMP 0, 0.31, 0.63, 1.3, 2.5, 5, 10 20 μ g
 1 μ M 가 40Ml MMP
 , 37 30 0.1M (pH 4.0) 가 , KON
 TRON SFM 25 328 393nm .

4 MT1 - MMP MMP - 2
 . MMP - 2() MT1 - MMP()
 (%) , 가

T1 - MMP 50% 2.0 μ g/M ℓ 0.82 μ g/M ℓ . MMP - 2
 , proMMP - 2 MMP - 2 sM
 , sMT1 - MMP MMP - 2

4 5 , sMT1 - MMP가 proMMP - 2 가
 2 MMP - 2 , proMMP - 2 MT1 - MMP proMMP -
 , MMP - 2

7: proMMP - 2

, MT1 - MMP APMA proMMP - 2 MMP - 2 ,
 proMMP - 2
 (coimmunoprecipitation) , proMMP - 2가
 proMMP - 2 .

1 μ g/M ℓ proMMP - 2 HT1080 0, 0.1, 1 10 μ g
 4 2 , proMMP - 2 , 25 μ ℓ
 FLAG (KODAK, U.S.A.)가 가 1 가 , 4000rpm
 30 , 0.5% NP - 40(Nonidet P - 40, Sigma, U.S.A.) 500 μ ℓ
 PBS 5 , MMP - 2 (Santa Cruz Biotechnology, U.S.A.) F
 LAG (western blotting) , MMP - 2

5a FLAG FLAG ,
 5b 가 MMP - 2 5a , 1
 가 HT1080 , 2 4
 0, 0.1, 1 10 μ g 가 HT1080 , 5b , H HT1080
 30 μ ℓ , 1 2 , 5a 1 4
 MMP - 2 . 5a 5b ,
 (20kDa) 가 , proMMP2(68kDa)가
 가 .

, proMMP - 2 MT1 - MMP proMMP - 2 M
 MP - 2 APMA proMMP - 2 MMP - 2 .

MT1 - MMP MMP - 2 (proMMP - 2)
 MMP - 2 ,

(57)

1.

(MMP - 2)

.

2.

1 ,

1

(MT1 - MMP)

(proMMP - 2)

(MMP - 2)

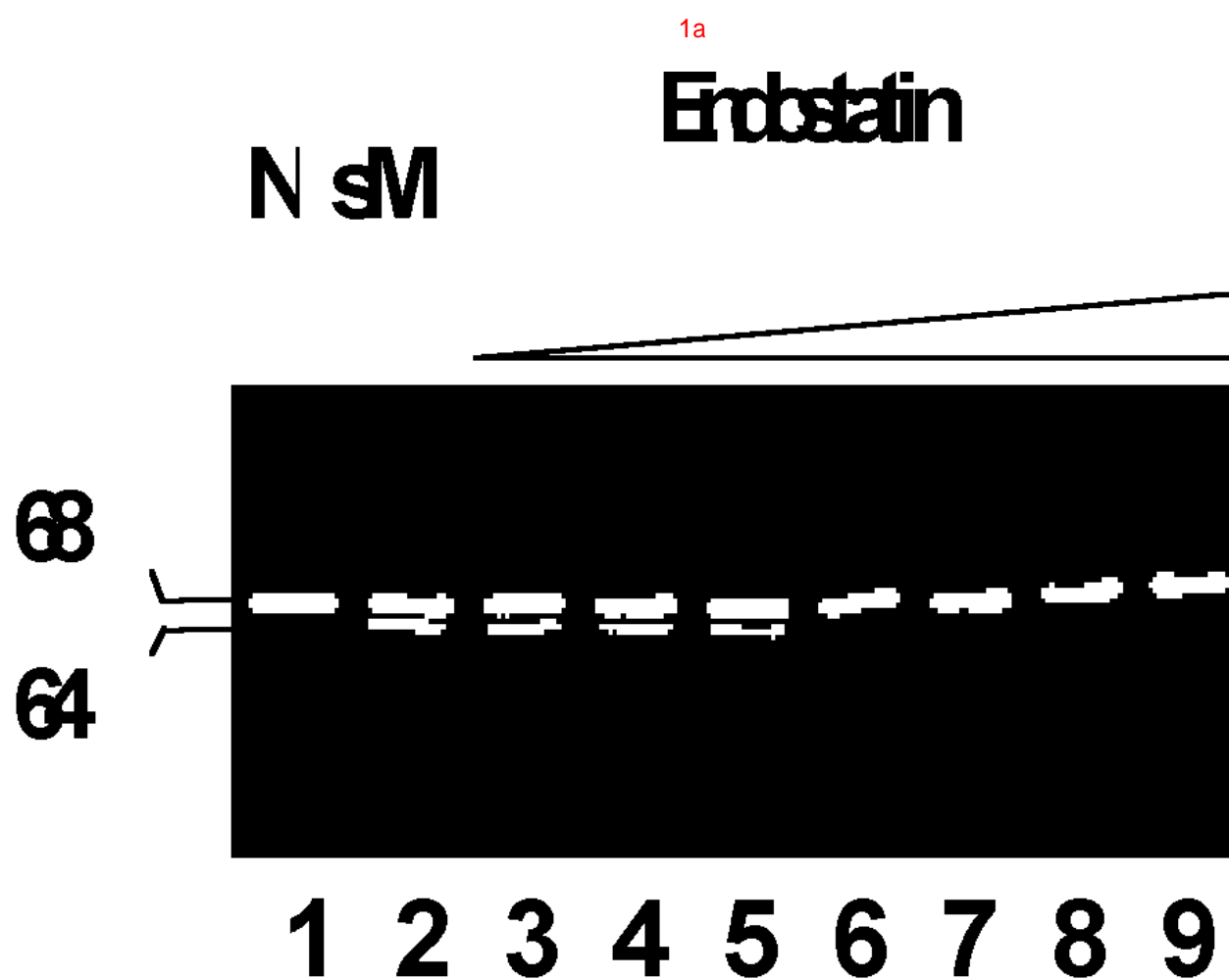
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3.

1 ,

(MMP - 2)

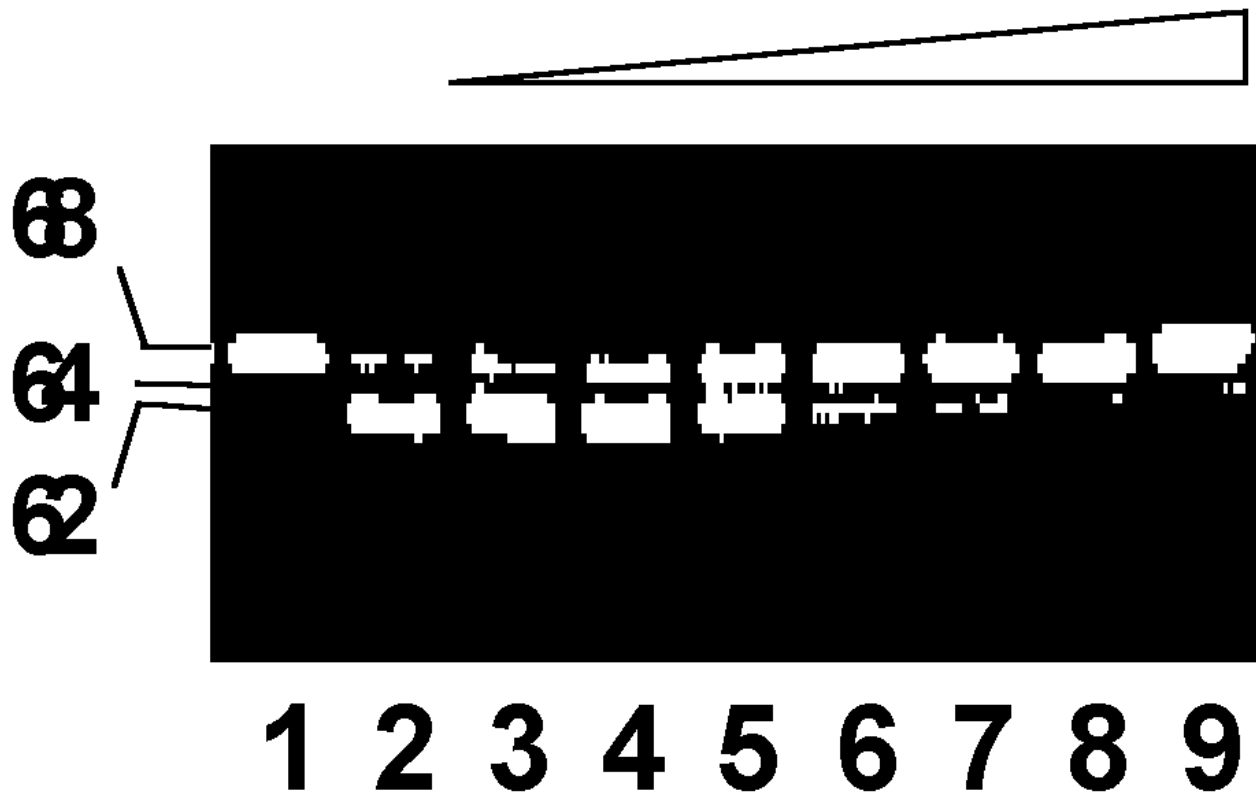
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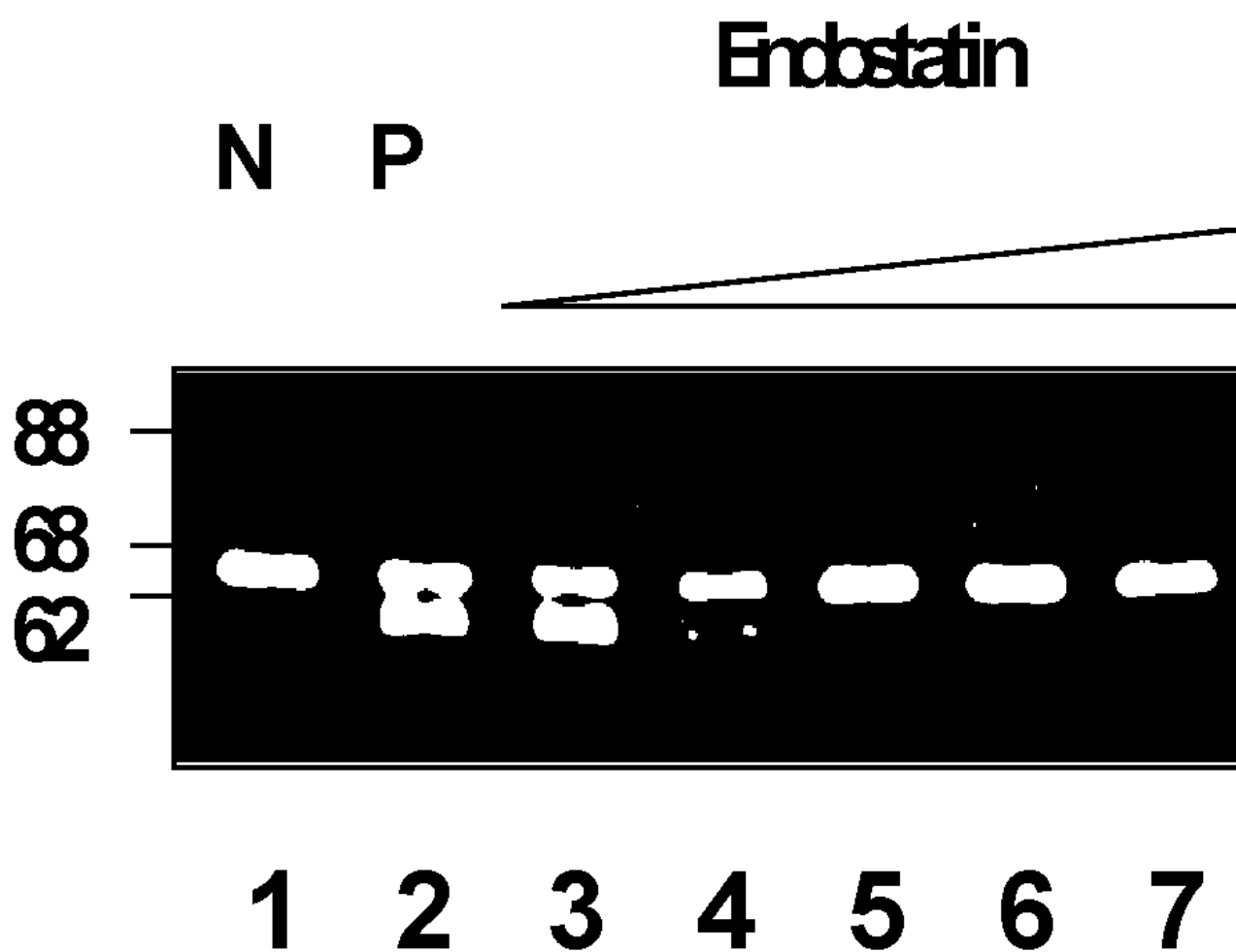
1b

Endostatin

N Am



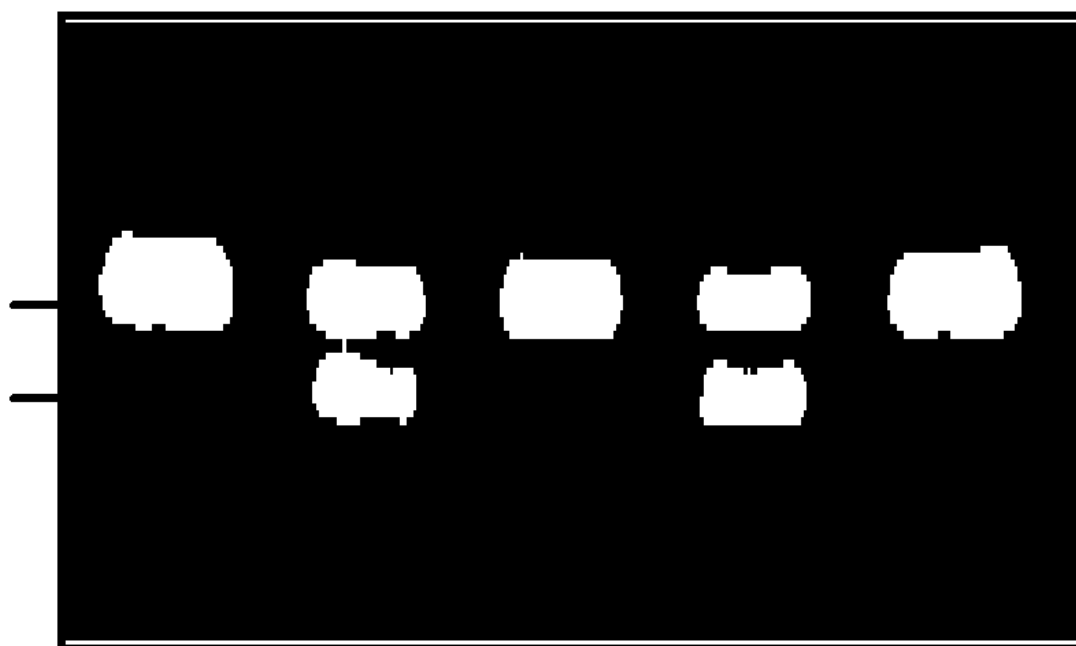
2



3a

1 2 3 4 5

68
62

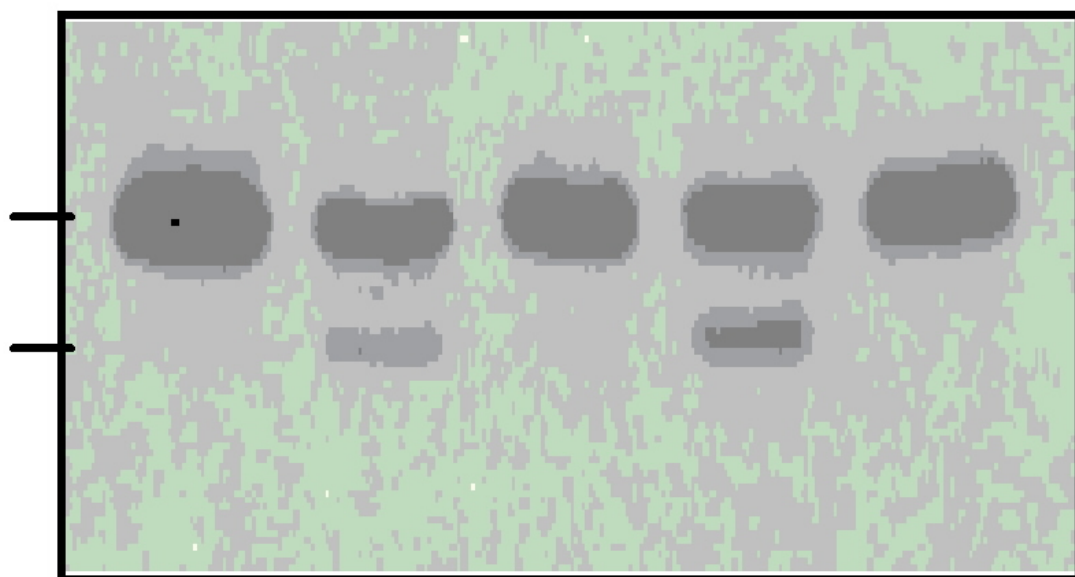


3b

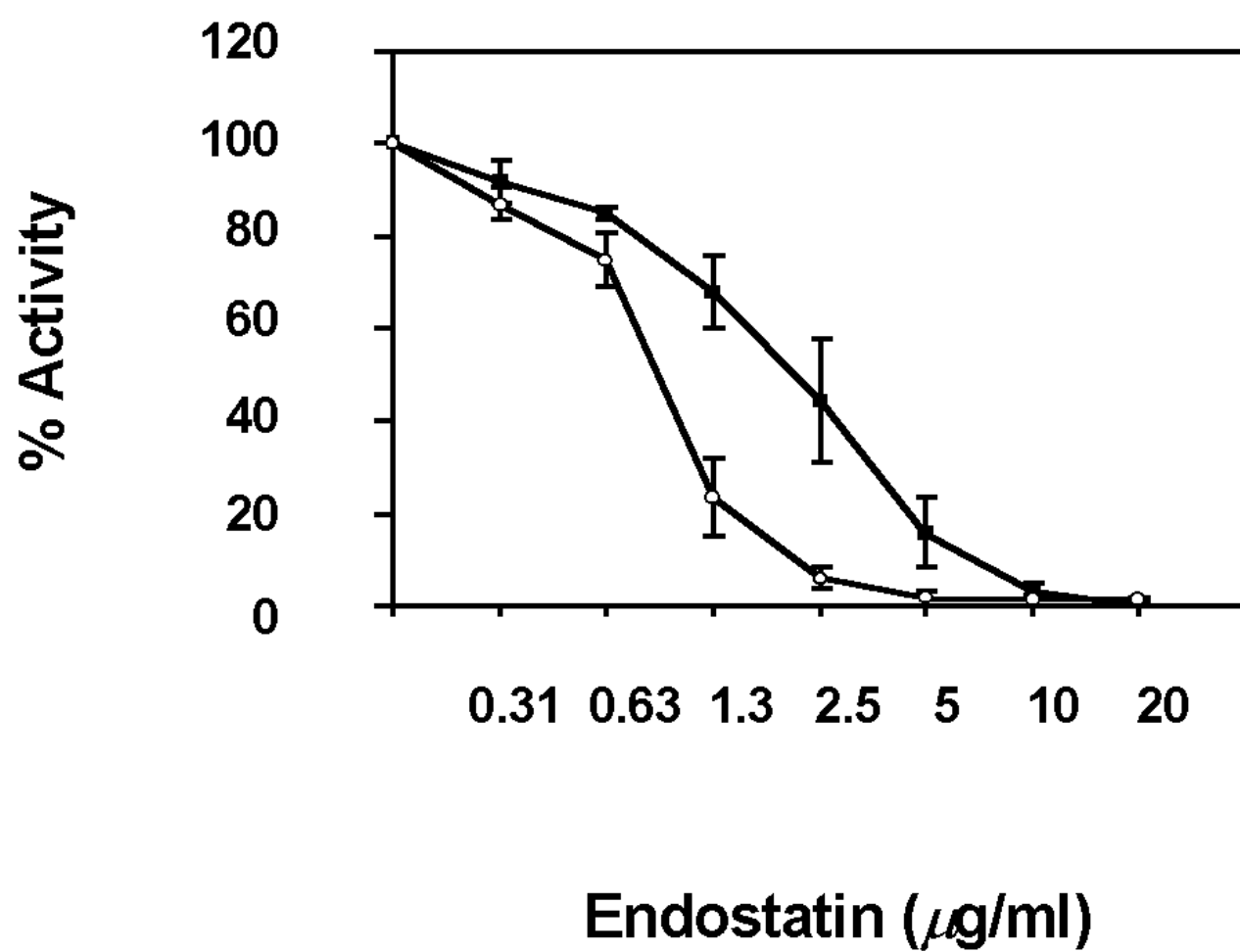
1 2 3 4 5

68

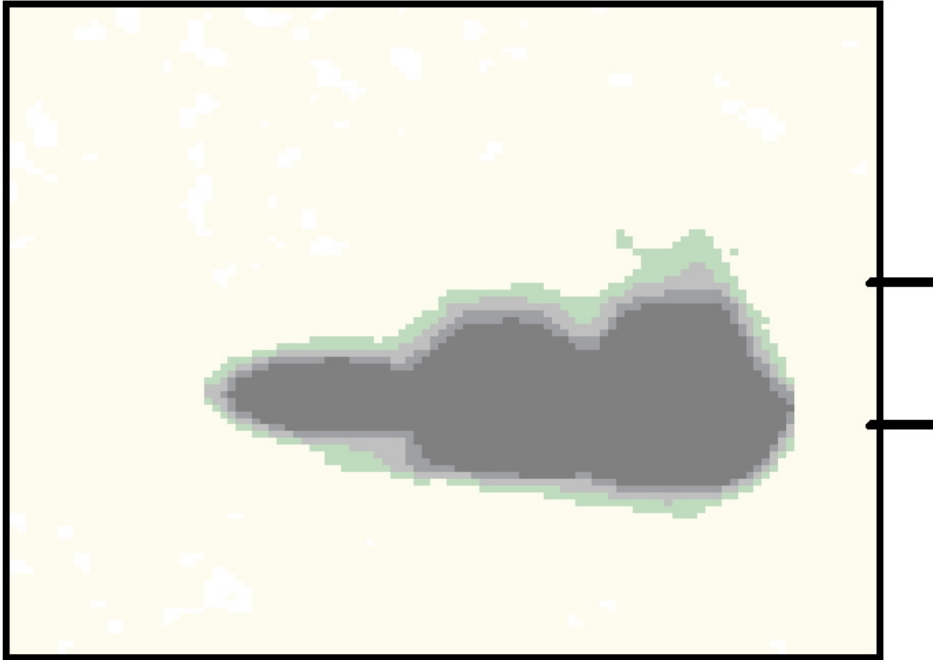
62



4



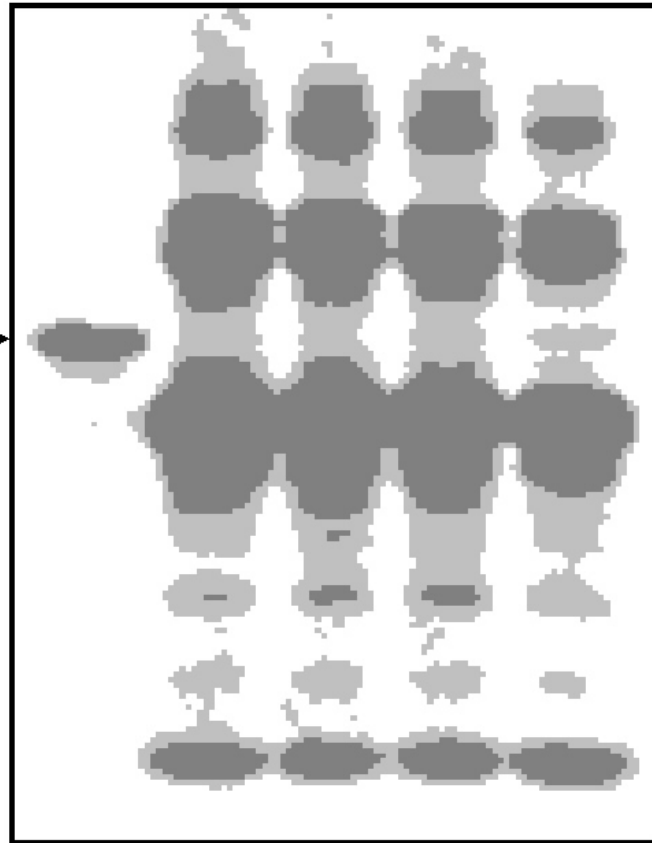
5a

1 2 3 4**29****21**

5b

H 1 2 3 4

proMMP2



<110> KWON, Young-Guen
 <120> A Method for Inhibiting Degradation of Extracellular Matrix by Endostatin through Inhibition of the Catalytic Activity of Matrix Metalloproteinases
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31