

APPENDIX A

List of chemical and materials used in this study

Name of chemicals	Company
Absolute ethanol	E.Merck, Germany
Acetic Acid	E.Merck, Germany
Acrylamide	Sigma-Aldrich, USA
Ammonium persulfate	Sigma-Aldrich, USA
β -actin antibody	Sigma-Aldrich, USA
Bovine serum albumin	Sigma-Aldrich, USA
Calcium chloride	Sigma-Aldrich, USA
Casein	Sigma-Aldrich, USA
Commasie brilliant blue R-250	Bio-Rad Laboratories, USA
Dimethyl sulfoxide(DMSO)	E.Merck, Germany
Dulbeco's modified eagel's medium	GIBCO, USA
EnzChek Gelatinase assay kit	Life Technologies Japan Ltd., Japan
Fetal bovine serum	Hyclone, USA
Gelatin	Bio-Rad Laboratories, USA
Glycine	Sigma-Aldrich, USA
HEPES	Sigma-Aldrich, USA

High range molecular weight marker	Bio-Rad Laboratories, USA
HRP-linked anti mouse IgG	GE Healthcare, UK
HRP-linked anti rabbit IgG	GE Healthcare, UK
Hydrochloric acid	E.Merck, Germany
Mercaptoethanol	Wako, Japan
Methanol	E.Merck, Germany
MTT	USB, USA
Non-fat dry milk	Wako, Japan
Plasminogen	Sigma-Aldrich, USA
Penicillin-streptomycin	GIBCO, USA
Potassium chloride	Sigma-Aldrich, USA
Potassium phosphate	Sigma-Aldrich, USA
Sephadex LH20	Sigma-Aldrich, USA
Sodium carbonate	Sigma-Aldrich, USA
Sodium chloride	Sigma-Aldrich, USA
Sodium dodecyl sulfate	Sigma-Aldrich, USA
Sodium hydroxide	Sigma-Aldrich, USA
Restore™ Western Blot stripping buffer	Thermo scientific, USA
Triton X-100	Sigma-Aldrich, USA
Trypsin-EDTA	GIBCO, USA
Tween 20	Sigma-Aldrich, USA

APPENDIX B

List of instrument used in this study

Instrument	Company
Analytical balance AC 100	Satorious
Autoclave	Tomy autoclave SS-240
Automatic pipette	GIBCO, Gilson
Bio-Max MR film	Eastman, Kodak, USA
Carbondioxide incubator	Forma Scientific
Compact Image-Analysis System (LAS-3000mini)	Fujifilm Life Science
Deionized water machine	Barnstead
Distilled water machine	Hamilton
Freezer (-80 °C)	Forma scientific
Freezer (-20 °C)	Sanyo
Glassware	Pyrex
Hood	British Klocker Switchgear
Hot air oven	Haraeus
ImageJ 1.410	National Institute of Mental Health, USA
Image Processor for Analytical Pathology (IPAP-WIN)	Sumika Technos, Japan
Inverted microscope	Nikon
Inertsil ODS-3-C18 columns	GL science Inc., Japan
Laminar flow biological cabinet	AIR2000 Fembrook Lane Plymouth, MN55447
Light microscope	Olympia Tokyo
Liquid nitrogen tank	Taylor-wharton

Lyophilizer	Christ Alpha1-4
Magnetic stirrer	Sybron / Thermolyne
Mastercycler® PCR machine	Eppendorf scientific
Microcentrifuge, bench-topped	Clay
Pasture pipette	Pyrex
pH meter	Hanna Instruments 8417
Power supply	E-CApparatus corporation
Refrigerator	Sanyo, Hitachi
Serological pipette	Pyrex
Shaker bath	Unitronic 320 OR
Sonicator	Sci Med
Spectrophotometer	MILTON ROY spectronic 1001
25 or 75 cm ³ T-flask	Nunc
6 or 24 or 24 well plate	Nunc
Trans-blot® electrophoretic transfer cell	Bio-Rad
Vortex	Scientific industries
Water bath	GFL 1083
YMC C30 column	Tokyo, Japan

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APPENDIX C

Preparation of some reagents and buffers

Cell Culture media

1. DMEM serum free medium with phenol red

DMEM	1	package (13.5 g)
HEPES	2.603	g
NaHCO ₃	3.7	g
0.34% 2-mercaptoethanol	1.0	ml

Add deionized water to 1000 ml and steriled by suction filter (membrane pore sized 0.2 μ m) and stored at 4 °C

2 DMEM with 10% fetal bovine serum

DMEM without serum free	89.5	ml
Fetal bovine serum	10	ml
Penicillin-streptomycin	0.5	ml

Stored at 4 °C

Measurement of cell survival

1. MTT stock dye solution

MTT	1	g
PBS pH 7.4	200	ml

Filtrate with membrane filter pore size 0.2 μ m, collect in dark container.

2. Phosphate buffer saline (PBS) pH 7.4

KH ₂ PO ₄	0.24	g
Na ₂ HPO ₄	1.44	g
NaCl	8.0	g
KCl	0.2	g

Dissolve in 800 ml deionized distilled water, adjust pH to 7.4 then top up to 1,000 ml.

Gelatin and casein-plasminogen Zymography

1. Stock solution A: separation gel buffer 1.5 mM Tris HCl, pH 8.8

Tris-base	18.15	g
Deionized water	80	ml

Adjust pH to 8.8 then adjust volume with deionized water to 100 ml and filtrated with 0.2 μ m membrane. Stored at 4 °C.

2. Stock solution C: stock acrylamide (30% w/v)

Acrylamide	29.2	g
Bis-acrylamide	0.8	g
Deionized water	70	ml

Adjust volume with deionized water to 100 ml and store at 4 °C

3. Stock Solution D: stacking gel buffer 0.5 mM Tris HCl pH 6.8

Tris-base	6.05	g
Deionized water	80	ml

Adjust pH to 6.8 then adjust volume with deionized water to 100 ml and filtrated with 0.2 μ m membrane. Stored at 4 °C.

4. Stock Ammonium persulfate solution (10%w/v)

Ammonium persulfate	100	mg
Deionized water	8	ml

Adjust volume with deionized water to 10 ml and store at -20°C

5. Electrode buffer

Tris-base	3	g
Glycine	14.4	g
SDS	1	g
Deionized water	800	ml

Adjust volume with deionized water to 1000 ml

6. 4X non reducing buffer

1.0 M Tris-HCl pH 6.8	625	μ l
Glycerol	1	ml

1% Bromphenol blue	125	µl
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Adjust volume with deionized water to 10 ml

7. Stock Gelatin (1%w/v)

Gelatin	100	g
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Deionized water	10	ml
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Stored at -20°C

8. Stock 10% SDS

SDS	2	g
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Deionized water	20	ml
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9. Stock Plasminogen (2mg/ml)

Plasminogen	2	g
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Deionized water	1	ml
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10. Separating gel 7.5% SDS- PAGE containing 0.1%(w/v) gelatin (gelatin zymography)

Deionized water	1.95	ml
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Solution A	3.75	ml
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10% SDS	0.05	ml
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Solution C	3.3	ml
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1%w/v gelatin	1	ml
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10% APS	0.05	ml
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TEMED	0.01	ml
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11. Separating gel 10% SDS-PAGE containing casein and plasminogen (casein zymography)

Casein	30	mg
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Deionized water	4	ml
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Solution A	2.5	ml
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10% SDS	0.1	ml
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Solution C	2.5	ml
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Plasminogen (2mg/ml)	0.05	ml
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10% APS	0.05	ml
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TEMED	0.01	ml
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12. Stacking gel 4%

Deionized water	3.05	ml
Solution D	1.25	ml
10% SDS	0.05	ml
Solution C	0.65	ml
10% APS	0.05	ml
TEMED	0.01	ml

13. 2.5% Triton X 100

Triton X-100	2.5	ml
Deionized water	97.5	ml

14. Activation Buffer

Tris-HCl	1.21	g
CaCl ₂	0.73	g
NaCl	2.92	g
Deionized water	800	ml

Adjust pH to 7.4 then adjust volume with deionized water to 1000 ml

Western blot analysis

1. RIPA contains proteinase inhibitor (prepare before used)

RIPA buffer	10	ml
Protease inhibitor cocktail kit	100	μl

2. Tris Buffered Saline (TBS)

Tris-base	2.42	ml
NaCl	8	ml
Deionized water	800	ml

Adjust pH to 7.4 then adjust volume with deionized water to 1000 ml

3. Separating gel 10% SDS-PAGE

Deionized water	2.66	ml
Solution A	3.75	ml
10% SDS	0.1	ml
Solution C	3.33	ml
10% APS	0.1	ml

TEMED	0.01	ml
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4. Stacking gel 4%

Deionized water	3.05	ml
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Solution D	1.25	ml
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10% SDS	0.05	ml
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Solution C	0.65	ml
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10% APS	0.05	ml
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TEMED	0.01	ml
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5. Blotting buffer

Tris-base	3.03	g
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Glycine	14.4	g
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Methanol	200	ml
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Deionized distilled water was top up to 1000 ml

6. TBS-T (0.1% v/v tween)

TBS	500	ml
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Tween	0.5	μl
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7. Blocking reagent (5% skim milk)

Skim milk	0.5	g
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TBS-T	100	g
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