SDS-PAGE sample preparation from tissue cultured cells

Protocol 1 (Direct RIPA method)

*example for 35mm dish, see the table for other dishs/flasks

Place dish on ice

Wash cells twice with 1ml of ice-cold PBS-STOP

*Only once for AD293 cells

Add RIPA1 directly to cells (200ul) and suspend cells

*For 10 cm dish, use a cell scraper to collect and transfer lysate.

Transfer cell lysate using cell scraper to a 1.5-mL tube

Incubate lysate on ice for 10min, occasional brief vortex (once or twice)

*Sample may be frozen and stored at this point at -80˚C

Centrifuge, 10min, 4˚C, 13krpm

Transfer sup to a new 1.5-mL tube (transfer 190ul)

Measure protein concentration using BCA protein assay kit (use 10ul for protein assay)

Add 3xSDS sample loading buffer (90ul)

Boil sample for 5min

Cool down sample on ice

Adjust protein concentration based on protein assay

Store sample at -20˚C

PBS STOP: Mix the following and store at 4˚C

385.5 mL MilliQ water

50 mL 10 x PBS

50 mL 0.5 M NaF

10 mL 0.5 M EDTA

2.5 mL 0.2 M NaN3

RIPA1

Ingredient	Stock soln.	To make 50ml
50mM Tris pH7.2	1M	2.5ml
150mM NaCl	5M	1.5ml

0.1% SDS	20%	0.25ml
0.5% DOC	10%	2.5ml
1% NP-40	10%	5ml
2mM EDTA	0.5M	0.2ml
50mM NaF	0.5M	5ml
H20		Measure up to 50ml

**Add 0.2 M (100 x) PMSF (final conc.: 2 mM or 1x) before use

**Add 100 x Halt (final 1x) before use

Amount of buffer/reagent used for this protocol

Dish size	6-well/35mm	60mm	100mm	T25
Cell number	Up to 5x10^5	Up to 1x10^6	Up to 4x10^6	Up to 1x10^6
RIPA1	200 ul	400 ul	700 ul	400 ul
Sup to save	160 ul	350 ul	630 ul	350 ul
Vol for BCA	10 ul	10 ul	10 ul	10 ul
3xSDS buffer	75 ul	170 ul	310ul	170ul

Protocol 2 (Scraping method)

*Example for 100 mm dish, see the table for other dishes (Not for flasks)

Place dish on ice

Wash cells twice with ice-cold PBS-STOP

Add 1 ml of PBS-STOP to dish

Transfer cells to a 1.5-mL tube using a cell scraper

Spin down cells by centrifugation, 1200 rpm, 3 min, 4˚C

Remove supernatant

*Cell pellet may be frozen at this point at -80˚C

Add 500ul of RIPA1 (ice-cold) to cell pellet

Suspend cells by pipetting

Incubate 10min, on ice to lyse cells, occasional brief vortex (one or twice)

Centrifuge, 10min, 4˚C, 13krpm

Transfer sup to new 1.5-mL tube (450ul)

Measure protein concentration using BCA protein assay kit (use 10 ul for protein assay)

Add 3xSDS sample loading buffer (220 ul)

Boil sample for 5min

Cool down sample on ice

Adjust protein concentration based on protein assay

Store sample at -20˚C

PBS STOP: Mix the following and store at 4˚C

385.5 mL MilliQ water

50 mL 10 x PBS

50 mL 0.5 M NaF

10 mL 0.5 M EDTA

2.5 mL 0.2 M NaN3

RIPA1

Ingredient	Stock soln.	To make 50ml
50mM Tris pH7.2	1M	2.5ml
150mM NaCl	5M	1.5ml
0.1% SDS	20%	0.25ml
0.5% DOC	10%	2.5ml
1% NP-40	10%	5ml
2mM EDTA	0.5M	0.2ml
50mM NaF	0.5M	5ml
H20		Measure up to 50ml

**Add 0.2 M (100 x) PMSF (final conc.: 2 mM or 1x) before use

**Add 100 x Halt (final 1x) before use

Amount of buffer/reagent used for this protocol

Dish size	6-well/35mm	60mm	100mm
Cell number	Up to 5x10^5	Up to 1x10^6	Up to 4x10^6
RIPA1	160 ul	300 ul	500ul
Sup to save	130 ul	260 ul	450ul
Vol for BCA	10 ul	10 ul	10 ul
3xSDS buffer	60 ul	125 ul	220ul

Protocol 3 (Trypsin method)

*Example for T25 flask, see the table for other dishes

Place flask on ice

Wash cells twice with ice-cold PBS-STOP

Add 1 ml of Trypsin to dish, tighten the cap

Incubate dish at RT or 37˚C until cells detach from flask surface

Suspend and transfer cells to a 15-mL tube that has 5 mL of ice-cold PBS-STOP

Spin down cells by centrifugation, 1200 rpm, 3 min, 4˚C

First wash: Remove supernatant and suspended cells with 5 mL of ice-cold PBS-STOP

Spin down cells by centrifugation, 1200 rpm, 3 min, 4˚C

Second wash: Remove supernatant and suspended cells with 5 mL of ice-cold PBS-STOP

Spin down cells by centrifugation, 1200 rpm, 3 min, 4˚C

*Cell pellet may be frozen at this point at -80˚C

Add 250ul of RIPA1 (ice-cold) to cell pellet

Suspend cells by pipetting

Incubate 10min, on ice to lyse cells, occasional brief vortex (one or twice)

Centrifuge, 10min, 4˚C, 13krpm

Transfer sup to new 1.5-mL tube (205ul)

Measure protein concentration using BCA protein assay kit (use 5 ul for protein assay)

Add 3xSDS sample loading buffer (100 ul)

Boil sample for 5min

Cool down sample on ice

Adjust protein concentration based on protein assay

Store sample at -20˚C

PBS STOP: Mix the following and store at 4˚C

385.5 mL MilliQ water

50 mL 10 x PBS

50 mL 0.5 M NaF

10 mL 0.5 M EDTA

2.5 mL 0.2 M NaN3

RIPA1

Ingredient	Stock soln.	To make 50ml
50mM Tris pH7.2	1M	2.5ml
150mM NaCl	5M	1.5ml
0.1% SDS	20%	0.25ml
0.5% DOC	10%	2.5ml
1% NP-40	10%	5ml
2mM EDTA	0.5M	0.2ml
50mM NaF	0.5M	5ml
H20		Measure up to 50ml

**Add 0.2 M (100 x) PMSF (final conc.: 2 mM or 1x) before use

**Add 100 x Halt (final 1x) before use

Amount of buffer/reagent used for this protocol

Dish size	6-well/35mm	60mm	100mm	T25
Cell number	Up to 5x10^5	Up to 1x10^6	Up to 4x10^6	Up to 1x10^6
RIPA1	120 ul	225 ul	500 ul	225 ul
Sup to save	105 ul	205 ul	460 ul	205 ul
Vol for BCA	5 ul	5 ul	10 ul	5 ul
3xSDS buffer	50 ul	100 ul	225 ul	100 ul