Cell Lysate Preparation

BETHYL LABORATORIES, INC

RIPA Method

Materials:

- Mammalian cells grown in adherent (100 mm dish) or suspension culture
- Ice cold RIPA Lysis Buffer
- Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X) (Thermo Scientific #78442)
- Ice cold PBS
- Ice

Recipes:

RIPA Lysis Buffer (store at 4°C up to 1 month)

Stock	Volume	[Final]
5 M NaCl	3 mL	150 mM
0.5 M EDTA, pH 8.0	1 mL	5 mM
1 M Tris, pH 8.0	5 mL	50 mM
NP-40 (IGEPAL CA-630)	1 mL	1.0%
10% sodium deoxycholate	5 mL	0.5%
10% SDS	1 mL	0.1%
dH2O	84 mL	

RIPA Lysis Buffer with Inhibitors (make fresh and keep on ice)

Stock	Volume	[Final]
Ice cold RIPA Lysis Buffer	10 mL	
100X Halt Protease Phosphatase Inhibitor CockTail	0.1 mL	1X

PBS (store at 4°C up to 1 month)

Ingredients		[Final]
NaCl	8.0 g	137 mM
KCl	0.20 g	2.7 mM
NaH2PO4	0.23 g	1.9 mM
Na2HPO4	0.12 g	0.8 mM
dH2O	1 L	

Procedures:

Adherent cells

1. Culture adherent cells to approximately 80% confluence on 100mm polystyrene tissue culture plates. Cells should be in log phase growth and healthy.
2. Aspirate or decant media and keep plates on ice for all steps.
3. Wash cell monolayer gently one time with 10 ml ice cold PBS. Aspirate excess PBS.
4. Add 200 to 500 µl of RIPA Lysis Buffer with Inhibitors to each plate and swirl to distribute buffer. *If harvesting multiple plates of the same cell type, 0.5 to 1 ml of Lysis Buffer can be used to sequentially lyse at least 5 plates; this results in a higher concentration of protein in the final lysate. The amount of lysis buffer should be empirically determined for each cell type to ensure efficient lysis as well as an optimal final concentration of protein in the lysate.*
5. Using a cell scraper or silicone spatula, scrape the cells and transfer the lysate to a 15 ml conical.
6. Incubate the lysate on ice for 15 minutes.
7. Sonicate the lysate (Branson Digital Sonifier set at 50% amplitude) three times for two seconds each with at least one minute rest on ice between each two-second pulse. If lysate is still viscous repeat sonication.
8. Incubate the lysate an additional 15 minutes.
9. Centrifuge at 13,000 x g for 5 minutes at 4°C.
10. Collect the supernatant (avoiding the pellet) into new microtubes.
11. Determine protein concentration by the bicinchoninic acid method (Pierce 23228).
12. Aliquot and store lysate at -20°C avoiding multiple freeze/thaw cycles.

Suspension culture

1. Culture cells to a density of 1-2 million cells/ml. Cells should be in log phase growth and healthy.
2. Pellet cells in a conical tube by spinning at 300 x g for 5 minutes at room temperature.
3. Aspirate or decant media; keep cells on ice for all steps.
4. Wash pellet one time with 5 to 10 ml ice cold PBS.
5. Spin 300 x g for 5 minutes. Decant the PBS wash and aspirate the excess supernatant.
6. Add 10 to 100 µl of RIPA Lysis Buffer with Inhibitors per 1 x 106 cells. *The amount of lysis buffer should be empirically determined for each cell type to ensure efficient lysis as well as an optimal final concentration of protein in the lysate.*
7. Incubate the lysate on ice for 15 minutes.
8. Sonicate the lysate (Branson Digital Sonifier set at 50% amplitude) three times for two seconds each with at least one minute rest on ice between each two-second pulse. If lysate is still viscous repeat sonication.
9. Incubate the lysate an additional 15 minutes.
10. Centrifuge at 13,000 x g for 5 minutes at 4°C.
11. Collect the supernatant (avoiding the pellet) into new microtubes.
12. Determine protein concentration by the bicinchoninic acid method (Pierce 23228).
13. Aliquot and store lysate at -20°C. Avoid multiple freeze/thaw cycles.

NETN Method

Materials:

- Mammalian cells grown in adherent (100 mm dish) or suspension culture
- Ice cold NETN Lysis Buffer
- Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (100X) (Thermo Scientific #78442)
- Ice cold PBS
- Ice

Recipes:

NETN Lysis Buffer (store at 4°C up to 1 month)

Stock	Volume	[Final]
5 M NaCl	5 mL	250 mM
0.5 M EDTA, pH 8.0	1 mL	5 mM
1 M Tris-HCl, pH 8.0	5 mL	50 mM
NP-40 (IGEPAL CA-630)	0.5 mL	0.5%
dH2O	88.5 mL	

NETN Lysis Buffer with Inhibitors (make fresh and keep on ice)

Stock	Volume	[Final]
Ice cold NETN Lysis Buffer	10 mL	
100X Halt Protease Phosphatase Inhibitor CockTail	0.1 mL	1X

PBS (store at 4°C up to 1 month)

Ingredients		[Final]
NaCl	8.0 g	137 mM
KCl	0.20 g	2.7 mM
NaH2PO4	0.23 g	1.9 mM
Na2HPO4	0.12 g	0.8 mM
dH2O	1 L	

Procedures:

Adherent cells

1. Culture adherent cells to approximately 80% confluence on 100mm polystyrene tissue culture plates. Cells should be in log phase growth and healthy.
2. Aspirate or decant media and keep plates on ice for all steps.
3. Wash cell monolayer gently one time with 10 ml ice cold PBS. Aspirate excess PBS.
4. Add 200 to 400 µl of NETN Lysis Buffer with Inhibitors to each plate and swirl to distribute buffer. *If harvesting multiple plates of the same cell type, 0.5 to 1 ml of Lysis Buffer can be used to sequentially lyse at least 5 to 7 plates; this results in a higher concentration of protein in the final lysate. The optimal volume of lysis buffer should be empirically determined for each cell type to ensure efficient lysis as well as an optimal final concentration of protein in the lysate.*
5. Using a cell scraper or silicone spatula, scrape the cells and transfer the lysate to a 15 ml conical using a 1 ml pipette and tip.
6. Incubate the lysate on ice for 30 minutes.
7. Centrifuge at 13,000 x g for 5 minutes at 4ºC.
8. Collect the supernatant (avoiding the pellet) into new microtubes.
9. Determine protein concentration by the bicinchoninic acid method (Pierce 23228).
10. Aliquot and store lysate at -20ºC avoiding multiple freeze/thaw cycles.

Suspension culture

1. Culture cells to a density of 1-2 million cells/ml. Cells should be in log phase growth and healthy.
2. Pellet cells in a conical tube by spinning at 300 x g for 5 minutes at room temperature.
3. Aspirate or decant media; keep cells on ice for all steps.
4. Wash pellet one time with 10 ml ice cold PBS.
5. Spin 300 x g for 5 minutes. Decant the PBS wash and aspirate the excess supernatant.
6. Add 10 to 100 µl of NETN Lysis Buffer with Inhibitors per 2 x 10^6 cells. *The optimal volume of lysis buffer should be empirically determined for each cell type to ensure efficient lysis as well as an optimal final concentration of protein in the lysate.*
7. Incubate the lysate on ice for 30 minutes.
8. Centrifuge at 13,000 x g for 5 minutes at 4ºC.
9. Collect the supernatant (avoiding the pellet) into new microtubes.
10. Determine protein concentration by the bicinchoninic acid method (Pierce 23228).
11. Aliquot and store lysate at -20ºC. Avoid multiple freeze/thaw cycles.

Nuclear Extract
Materials:

- Mammalian cells (100 mm dish, adherent culture)
- Ice
- Cold PBS
- Cold Buffer A
- Cold Buffer B
- Halt protease and phosphatase inhibitor single-use cocktail (100X) (Thermo Scientific #78442)
- 10% IGEPAL CA-630
- 1 M Dithiothreitol (DTT)

Recipes:
Buffer A (enough for 10 plates; store at 4°C up to 1 month)

Stock	Volume	[Final]
1 M HEPES, pH 7.9	50 µl	10 mM
1 M KCL	50 µl	10 mM
0.5 M EDTA	1 µl	0.1 mM
dH2O	4.889 ml	

Buffer B (enough for 10 plates; store at 4°C up to 1 month)

Stock	Volume	[Final]
1 M HEPES, pH 7.9	40 µl	20 mM
5 M NaCl	160 µl	0.4 M
0.5 M EDTA	4 µl	1.0 mM
Glycerol	200 µl	10%
dH2O	1.596 ml	

Procedures:

1. Culture adherent cells to approximately 80% confluence on 100mm polystyrene tissue culture plates. Cells should be in log phase growth and healthy.
2. Add the following to 5 ml Buffer A:
3. 50 µl Halt protease and phosphatase inhibitors
4. 200 µl 10% IGEPAL CA-630
5. 5 µl 1M DTT
6. Aspirate or decant media
7. Wash cell monolayer gently twice with 10 ml cold PBS. Aspirate or decant excess PBS
8. Add 0.5 ml of Buffer A with inhibitors, IGEPAL, and DTT to each plate and swirl to distribute buffer
9. Incubate at room temperature (RT) for 10 minutes
10. Using a cell scraper or silicone spatula, scrape the cells and pipet up and down with P1000 several times to disrupt cell clumps
11. Transfer the lysate to 1.5 ml microcentrifuge tubes
12. Centrifuge at 4°C at top speed (15,000 X *g*) for 3 minutes
13. Remove supernatant. Save supernatant (cytosolic fraction), if desired; otherwise, discard
14. Add the following to 2 ml of Buffer B:
15. 20 µl Halt protease and phosphatase inhibitors
16. 2 µl DTT
17. Re-suspend each pellet by adding 150 µl of Buffer B with inhibitors and DTT. Pipette up and down with a P200
18. Place the tubes on ice for 2 hours. During the two hour incubation, vortex the tubes every 15 minutes
19. Centrifuge at 4°C at top speed (15,000 X *g*) for 5 minutes
20. Remove and pool supernatants into a fresh tube
21. Determine protein concentration by the bicinchoninic acid method (Pierce 23228) or other preferred method
22. Aliquot and store extract at -80°C or -20°C avoiding multiple freeze/thaw cycles