FORTIS®
LIFE SCIENCES

Protocols for Cell Lysate Preparation

BETHYL
A FORTIS LIFE SCIENCES COMPANY

FORTIS LIFE SCIENCES

INTRODUCTION

This eBook is a comprehensive resource for cell lysate preparation. In this eBook, you will learn how to select the right buffer for your intended application. You will also find in-depth information about four common methods for cell lysis, including recipes for the required buffer preparation and protocols to guide your experimental design. Finally, discover antibodies that can be used following cell lysate preparation to continue your experiment with immunoprecipitation or western blot.

CONTENTS

BETHYL
A FORTIS LIFE SCIENCES COMPANY

Cell Lysate Preparation - RIPA Method

RIPA (Radioimmunoprecipitation Assay) Buffer is a reagent used in cell lysis experimentation, to enable rapid, efficient solubilization of proteins. By minimizing non-specific protein binding, specific binding interactions can be easily studied and are commonly used in immunoprecipitation experiments.

Materials

- Mammalian cells grown in adherent (100 mm dish) or suspension culture
- Ice cold RIPA Lysis Buffer
- Protease and Phosphatase Inhibitor Cocktail (100x)
- 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%
dH_2O	84 mL	

RIPA Lysis Buffer with Inhibitors

Make fresh and keep on ice

Stock	Volume	[Final]
Ice cold RIPA Lysis Buffer	10 mL	
Protease and Phosphatase Inhibitor (100x)	0.1 mL	1X

PBS

Store at 4°C up to 1 month

Ingredients	Volume	[Final]
NaCl	8.0 g	137 mM
KCl	0.20 g	2.7 mM
NaH_2PO_4	0.23 g	1.9 mM
Na_2HPO_4	0.12 g	0.8 mM
dH_2O	1 L	

Protocol

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 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 4ºC.
10. Collect the supernatant (avoiding the pellet) into new microtubes.
11. Determine protein concentration by the bicinchoninic acid method.
12. 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 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 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 4ºC.
11. Collect the supernatant (avoiding the pellet) into new microtubes.
12. Determine protein concentration by the bicinchoninic acid method.
13. Aliquot and store lysate at -20ºC. Avoid multiple freeze/thaw cycles.

Cell Lysate Preparation - NETN Method

NETN is a mild lysis buffer that is useful for separating the nuclear and cytoplasmic fractions of cell lysate.

Materials

- Mammalian cells grown in adherent (100 mm dish) or suspension culture
- Ice cold NETN Lysis Buffer
- Protease and Phosphatase Inhibitor Cocktail (100X)
- 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%
dH_2O	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 Protease and Phosphatase Inhibitor Cocktail	0.1 mL	1X

PBS

Store at 4°C up to 1 month

Ingredients	Volume	[Final]
NaCl	8.0 g	137 mM
KCl	0.20 g	2.7 mM
NaH_2PO_4	0.23 g	1.9 mM
Na_2HPO_4	0.12 g	0.8 mM
dH_2O	1 L	

Protocol

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 4°C.
8. Collect the supernatant (avoiding the pellet) into new microtubes.
9. Determine protein concentration by the bicinchoninic acid method.
10. 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 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 106 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 4°C.
9. Collect the supernatant (avoiding the pellet) into new microtubes.
10. Determine protein concentration by the bicinchoninic acid method.
11. Aliquot and store lysate at -20°C. Avoid multiple freeze/thaw cycles.

Cell Lysate Preparation - Nuclear Extract

Extracts prepared from the isolated nuclei of cultured cells can be used in functional studies and for the purification of proteins. This protocol outlines the steps and processes required.

Materials

- Mammalian cells (100 mm dish, adherent culture)
- Ice
- Cold PBS
- Cold Buffer A
- Cold Buffer B
- Protease and Phosphatase Inhibitor Cocktail (100x)
- 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
dH_2O	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 (252 mg)	10%
dH_2O	1.596 mL	

Protocol

Culture adherent cells to approximately 80% confluence on 100mm polystyrene tissue culture plates. Cells should be in log phase growth and healthy.

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

Cell Lysate Preparation - for Immunoprecipitation

This protocol outlines the preparation of cell lysate used in immunoprecipitation, with the required reagents and the step-by-step procedure.

Recipes

PBS

NaCl	8.0 g
KCl	0.2 g
NaH_2PO_4	0.23 g
Na_2HPO_4	0.12 g

Adjust the volume to 1 liter with distilled water.
Store at 4-25°C.

IP Lysis Buffer

NaCl	7.31 g
1 M Tris, pH 8	25 mL
0.5 M EDTA, pH 8	5 mL
10% NP-40	25 mL
Distilled Water	445 mL

Store at 4°C.

Protocol

1. Allow cells to grow to approximately 80% confluence on 100mm polystyrene tissue culture plates. Approximately 8*106 cells (one plate at 80% confluence) are needed per IP reaction.
2. Wash cells two times in cold PBS.
3. Lyse cells to release soluble cellular proteins using 500 mcl of cold lysis buffer containing 1X protease inhibitors per 100 mm plate. Scrape cells from the plates, transfer to 1.5 mL micro-centrifuge tubes, and place on ice for 30 minutes to ensure efficient lysis.
4. Centrifuge lysates at 10,000 x g; 5 minutes.
5. Remove and pool the supernatant. Determine protein concentration.

FORTIS®
LIFE SCIENCES

Find antibodies for your experiment

The protocols described in this eBook are primarily used to prepare for immunoprecipitation and western blot experiments. Bethyl Laboratories, a Fortis Life Sciences® company, offers nearly 7,000 antibodies validated for these applications across research areas including immunology, oncology, neuroscience, cell biology, and more. Use the links below to browse for antibodies that meet your needs.

Immunoprecipitation

Discover nearly 5000 antibodies validated for use in immunoprecipitation experiments.

Western blot

Find over 5500 antibodies validated for western blotting.

BETHYL
A FORTIS LIFE SCIENCES COMPANY