Whole cell lysates from tissue culture cells

Whole cell lysates are relatively simple and rapid to generate from adherent and suspension tissue culture cells—or even tissue slices—when a biochemical activity or cellular component cannot be easily examined in vivo and needs to be reconstituted or interrogated in vitro.

Results often depend on detergent composition and stringency of the lysis buffer. RIPA lysis buffer (*Alternative A*) is a strong lysis buffer suitable for extracting a wide range of proteins from various cellular compartments. NP-40 lysis buffer (*Alternative B*) is milder and designed primarily to extract cytoplasmic proteins under non-denaturing conditions. Urea lysis buffer (*Alternative C*) is optimized for proteome analysis by mass spectrometry.

While many researchers assume that their chosen method for total protein extraction faithfully reflects the protein composition in vivo, few rigorously compare different protocols to validate this assumption. Moreover, whole cell lysis inherently destroys subcellular compartmentalization, which can lead to artifactual interactions between proteins that are normally separated. Consequently, findings obtained from whole cell lysates should be critically evaluated in the context of these limitations.

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

□ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE

Reviewed: Feb 22, 2025

Procedures

›› ***Choosing a suitable lysis buffer***

(1.) Select a lysis buffer based on the target protein class and intended downstream application. The table below summarizes the solubility of representative protein classes in each buffer.

Protein	RIPA	NP-40	Other
Whole cell extracts	Good	Recommended	
Cytosolic proteins	Good	Good	Tris-HCl
Nuclear proteins	Recommended	Limited	
Mitochondrial proteins	Recommended	Limited	
Membrane-bound proteins	Recommended	Good	
High molecular weight proteins	Poor	Poor	Urea
Structural proteins: Fibronectin, Keratin, Lamin, Tubulin	Limited	Poor	Tris-Triton
Signaling proteins: EGFR, HSP90, c-Src	Poor	Poor	
Heterochromatin: H3K4me2	Insoluble	Insoluble	Acid extraction
Transcription factors: GATA2	Insoluble	Insoluble	
Apoptotic markers: Cleaved Caspase-8	Insoluble	Insoluble	

Hint: In cases where protein solubility is poor, the specimen can be taken up directly in Laemmli sample buffer to analyze protein recovery by immunostaining. However, the high SDS content of this buffer interferes with other applications such as immunoprecipitation of protein complexes and many enzymatic assays.

›› *Preparation of cell lysates*

- ☐ Phosphate-buffered saline, pH 7.4
- ☐ 100 × Inhibitor cocktail

(1.) Start with 1×10^6–1×10^9 cells. Approximate the packed cell volume; this is 1 vol. Typically, 1×10^9 cells will amount to 1–2 mL.

Critical: Keep buffer volumes to a minimum to maintain high protein concentrations.

Note: There is no need to thaw frozen cell pellets.

(2.) *For suspension cells:* Wash the cells twice with 5 vol ice-cold PBS containing the desired inhibitors. Collect the cells by centrifugation at 400 × *g* for 5 min at 4 °C after each step. Resuspend in lysis buffer.

For adherent cells: Wash the cells twice with ice-cold PBS containing the desired inhibitors. Aspirate the growth medium and residual wash buffer.

Apply lysis buffer directly to the plate and scrape cells using a cold plastic cell scraper. Transfer lysate to a fresh tube.

For tissues: Weigh out the tissue specimen, cut into pieces on ice, transfer to a round-bottom microcentrifuge tube. Freeze by immersing in liquid nitrogen. Homogenize in lysis buffer on ice.

(3.) *Optional:* Sonicate the lysate twice for 10 s at 20 kHz on ice to disrupt cellular membranes, shear genomic DNA, and aid with protein solubilization.

Critical: Since sonication can damage proteins, minimize pulse length and number of pulses. Apply short pulses and lower the power level to avoid frothing.

Hint: Alternatively, use a universal nuclease to enzymatically digest DNA and RNA. Use 25 U per 1 mL of cell lysate; incubate for 15 min at room temperature.

A › *Protein extraction with RIPA lysis buffer*

- ☐ Radioimmunoprecipitation lysis buffer, 50 mL ⟨*R*⟩

(1.) Add 50–100 vol ice-cold 1 × RIPA lysis buffer with inhibitors, about 1 mL per 1×10^7 cells or 5 mg of tissue. Agitate the suspension for 20 min at 4 °C. 20 min

Critical: Tissue samples will take 1–2 h or longer to complete lysis. Homogenize with an electric homogenizer or mortar.

(2.) Remove the insoluble fraction by centrifugation at 12 000 × *g* for 20 min at 4 °C. 20 min

(3.) Carefully collect the supernatant containing the soluble protein fraction in a new tube on ice.

B › *Protein extraction with NP-40 lysis buffer*

- ☐ NP-40 lysis buffer, 50 mL ⟨*R*⟩

(1.) Add 50–100 vol ice-cold 1 × NP-40 lysis buffer with inhibitors, about 1 mL per 1×10^7 cells or 5 mg of tissue. Agitate the suspension for 20 min at 4 °C. 20 min

Critical: Tissue samples will take 1–2 h or longer to complete lysis. Homogenize with an electric homogenizer or mortar.

(2.) Remove the insoluble fraction by centrifugation at 12 000 × *g* for 20 min at 4 °C. 20 min

(3.) Carefully collect the supernatant containing the soluble protein fraction in a new tube on ice.

C › ***Proteome extraction***

- ☐ Urea lysis buffer, 50 mL ⟨*R*⟩

(1.) Add 5 vol ice-cold 1 × urea lysis buffer with inhibitors, about 100 µL per $1{\times}10^7$ cells or 5 mg of tissue. Pipette the sample up and down to break up cell clumps and vortex gently.

(2.) Boil the lysate at 95 °C for 5 min.

(3.) *Optional:* Sonicate the lysate twice for 10 s at 20 kHz on ice to disrupt cellular membranes, shear genomic DNA, and aid with protein solubilization.

> ***Critical:*** Since sonication can damage proteins, minimize pulse length and number of pulses. Apply short pulses and lower the power level to avoid frothing.

> ***Hint:*** Alternatively, use a universal nuclease to enzymatically digest DNA and RNA. Use 25 U per 1 mL of cell lysate; incubate for 15 min at room temperature.

(4.) Remove the insoluble fraction by centrifugation at 12 000 × *g* for 20 min at 4 °C. ⏲ 20 min

(5.) Carefully collect the supernatant containing the soluble protein fraction in a new tube on ice.

Analyses

○ Determine the total protein concentration against 1 × lysis buffer.

○ Prepare samples for SDS-PAGE SOP 0007 corresponding to $2{\times}10^5$ cells (or 20 µg protein) for the soluble and for the insoluble fraction of the whole-cell extract. Immunostain for the protein of interest.

Troubleshooting

Choosing a suitable lysis buffer

In Step 1:

– Low protein yield
  - Verify cell count and ensure sufficient lysis buffer volume. Use at least 50–100 packed-cell volumes of buffer.
  - Extend lysis time or add sonication to improve solubilization of membrane and nuclear proteins.
– High viscosity; lysate is stringy or difficult to pipette
  - Shear genomic DNA by sonication or treat with a universal nuclease such as Benzonase® Nuclease.
– Protein degradation visible as unexpected low-MW bands or loss of signal
  - Add fresh protease inhibitor cocktail immediately before lysis. Keep all steps at 4 °C. Ensure samples are not left on ice for extended periods without inhibitors.
– Target protein not detected in the soluble fraction
  - Check the insoluble pellet by resuspending directly in Laemmli sample buffer. If the target is present in the pellet, increase detergent stringency or switch to a urea-based buffer.

Recipes

Radioimmunoprecipitation lysis buffer (RIPA), pH 7.6

Amount	Ingredient		Stock	Final
2.5 mL	Tris-Cl, pH 7.4	R0055	1 M	50 mM
1.5 mL	Sodium chloride (NaCl)	R0046	5 M	150 mM
500 µL	IGEPAL® CA-630	[9002-93-1]	250.38 g/mol	1.0%
250 mg	Sodium deoxycholate	[302-95-4]	414.55 g/mol	0.5%
250 µL	Sodium dodecylsulfate (SDS)	R0047	20%	0.1%
100 µL	EDTA, pH 8.0	R0017	0.5 M	1 mM
10 µL	Dithiothreitol (DTT) □	R0015	1 M	2 mM
To 50 mL	Water, reagent-grade			

Filter sterilize. Add reducing agent right before use. Can be aliquoted and stored at −20 °C. Store at 4 °C. ***Note:*** This is Proteintech's optimized RIPA buffer recipe. ***This is why:*** IGEPAL® CA-630 is a mild detergent alternative to Nonidet P-40 which is no longer manufactured; it can be replaced with Triton™ X-100.

Radioimmunoprecipitation lysis buffer (RIPA)

50 mM Tris-Cl, 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.5% Sodium deoxycholate, 0.1% SDS, 1 mM EDTA, □ 2 mM DTT, pH 7.6

WARNING

Eye irritation

Date: Sign: R0148

NP-40 lysis buffer, pH 7.4

Amount	Ingredient		Stock	Final
2.5 mL	Tris-Cl, pH 7.4	R0055	1 M	50 mM
1.5 mL	Sodium chloride (NaCl)	R0046	5 M	150 mM
500 µL	IGEPAL® CA-630	[9002-93-1]	250.38 g/mol	1.0%
10 µL	Dithiothreitol (DTT) □	R0015	1 M	2 mM
100 µL	EDTA, pH 8.0	R0017	0.5 M	1 mM
To 50 mL	Water, reagent-grade			

Filter sterilize. Add reducing agent right before use. Can be aliquoted and stored at −20 °C. Store at 4 °C. ***Note:*** Add up to 1% sodium deoxycholate to increase stringency. ***This is why:*** IGEPAL® CA-630 is a mild detergent alternative to Nonidet P-40 which is no longer manufactured; it can be replaced with Triton™ X-100.

NP-40 lysis buffer

50 mM Tris-Cl, 150 mM NaCl, 1.0% IGEPAL® CA-630, □ 2 mM DTT, 1 mM EDTA, pH 7.4

WARNING

Eye irritation

Date: Sign: R0149

Tris-Triton buffer, pH 7.4

Amount	Ingredient		Stock	Final
500 µL	Tris-Cl, pH 7.4	R0055	1 M	10 mM
1.0 mL	Sodium chloride (NaCl)	R0046	5 M	100 mM
500 µL	Triton™ X-100	R0057	10%	1.0%
250 mg	Sodium deoxycholate	[302-95-4]	414.55 g/mol	0.5%
250 µL	Sodium dodecylsulfate (SDS)	R0047	20%	0.1%
5.0 mL	Glycerol	R0022	50%	10%
10 µL	Dithiothreitol (DTT) □	R0015	1 M	2 mM
To 50 mL	Water, reagent-grade			

Filter sterilize. Add reducing agent right before use. Can be aliquoted and stored at −20 °C. Store at 4 °C.

Tris-Triton buffer

10 mM Tris-Cl, 100 mM NaCl, 1.0% Triton™ X-100, 0.5% Sodium deoxycholate, 0.1% SDS, 10% Glycerol, □ 2 mM DTT, pH 7.4

WARNING

Eye irritation

Date: Sign: R0150

Urea lysis buffer, pH 7.4

Amount	Ingredient		Stock	Final
10 mL	HEPES, pH 7.5	R0024	1 M	200 mM
27.0 g	Urea, ultrapure	[57-13-6]	60.06 g/mol	9 M
To 50 mL	Water, reagent-grade			

Reconstitute urea in a water bath of 25–30 °C for 30 min to aid dissolution.
Note: Keep at room temperature to avoid precipitation.

Urea lysis buffer

200 mM HEPES, 9 M Urea, pH 7.4

WARNING

Eye irritation; Skin irritation

Date: Sign: R0151

Change log

2020-03-22	Shany Koren-Hauer	Initial version.
2025-02-22	Benjamin C. Buchmuller	Adaptation as SOP.