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 intact cells (in cellulo) or living organisms (in vivo) or needs to be reconstituted or interrogated in vitro.

Success often depends 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 used 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 specimen's protein composition, 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. Where compartmentalization matters, isolate and fractionate first. Lysates destined for immunoprecipitation continue with the protocol for soluble proteins SOP 0027; chromatin-bound targets are not lysed here but solubilized as nucleosomes for the native chromatin protocol SOP 0047.

Risk assessment

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

▷ Wear gloves, safety glasses, lab coat

□ Dispose waste after inactivation as REGULATED MEDICAL WASTE

Reviewed: Sep 7, 2026

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 source	RIPA	NP-40	Other extraction methods or buffers
Whole cell extracts	Good	Recommended	
Cytosolic proteins	Good	Good	Tris-HCl
Nuclear proteins	Recommended	Limited	Nuclear extraction SOP 0025
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	
Apoptotic markers: Cleaved Caspase-8	Insoluble	Insoluble	
Transcription factors: GATA2	Insoluble	Insoluble	Nuclear extraction SOP 0025
Heterochromatin: HP1, H3K9me3	Insoluble	Insoluble	MNase digestion SOP 0025
Histones: H3, H4	Insoluble	Insoluble	Acid extraction SOP 0026, or as intact nucleosomes SOP 0025-A for immunoprecipitation SOP 0047

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 most enzymatic assays.

›› *Preparation of cell lysates*

- ☐ Phosphate-buffered saline, pH 7.4 ◇ R0037
- ☐ 100 × Inhibitor cocktail ◇ R0090
- ☐ Cell scraper, plastic
- ☐ Microcentrifuge tube, round-bottom
- ☐ Sonicator (*optional*)

(1.) Start with 1×10^6–1×10^9 cells, or with a weighed tissue specimen of 20–50 mg, depending on the downstream application.

Downstream application	Total protein	Total cells	Lysis buffer
Immunoblot, 20–50 µg per lane	0.2–0.5 mg	1×10^6–3×10^6	0.1–0.3 mL
Immunoprecipitation	0.5–2.0 mg	5×10^6–1×10^7	0.5–1.0 mL
Proteomics, activity assays	1.0–5.0 mg	1×10^7–5×10^7	1.0–5.0 mL

This is why: Mammalian tissue culture cells yield about 150–250 pg protein and occupy about 1.5 pL packed volume per cell. Soft tissue yields about 100–150 µg protein per milligram wet weight. At 1×10^8 cells and above, measure the packed cell volume instead and use 50–100 vol of RIPA or NP-40 lysis buffer, or half that of urea lysis buffer.

Critical: Keep buffer volumes to a minimum to maintain protein concentration at 1–5 mg/mL. Below 0.5 mg/mL, immunoprecipitation and activity assays starve for target; above 10 mg/mL, lysis is incomplete and the lysate becomes viscous.

(2.) Wash the cells twice with ice-cold PBS containing the desired inhibitors and bring the material into lysis buffer, depending on the specimen:

- ○ Collect suspension cells at 400 × *g* for 5 min at 4 °C, resuspend the pellet in lysis buffer.
- ○ For adherent cells, aspirate the last wash completely, apply lysis buffer directly to the dish, scrape with a cold plastic cell scraper, transfer the lysate to a fresh tube.
- ○ Weigh the tissue specimen, cut it into pieces on ice in a round-bottom microcentrifuge tube, snap-freeze in liquid nitrogen, and homogenize in lysis buffer on ice. Homogenize larger specimen with an electric homogenizer or mortar.

Hint: Always apply protease inhibitor cocktail. Add phosphatase and deacetylase inhibitors when phosphorylation or acetylation are assessed. Add them to the harvest buffer, not to living cells: phosphatase and deacetylase inhibitors induce the marks they are meant to preserve.

Note: Both fresh and frozen materials can be used. There is no need to thaw frozen cell pellets.

(3.) If the lysate is viscous or membrane and nuclear proteins extract poorly, reduce the viscosity and aid solubilization by removing nucleic acids in one of the following ways:

- ○ Sonicate the lysate twice for 10 s at 20 kHz on ice.

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

- ○ Add a universal nuclease at 25 U per 1 mL of lysate and incubate for 15 min at room temperature.

  Critical: The nuclease requires magnesium, and the RIPA and NP-40 lysis buffers carry 1 mM EDTA. Supplement the lysate with $MgCl_2$ to 2 mM before adding the enzyme.

A › *Protein extraction with RIPA lysis buffer*

- ☐ Radioimmunoprecipitation lysis buffer, pH 7.6 ◇ R0148

(1.) Add ice-cold 1 × RIPA lysis buffer with inhibitors, 100 µL per 1×10^6 cells or per 2 mg tissue, sized per the guide in the preparation step. Agitate the suspension for 20 min at 4 °C. ⏲ 20 min

Critical: Tissue samples will take 1–2 h or longer to complete lysis.

(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, pH 7.4 ◇ R0149
  - *or*: Tris-Triton buffer, pH 7.4 ◇ R0150

(1.) Add ice-cold 1 × NP-40 lysis buffer with inhibitors, 100 µL per $1{\times}10^6$ cells or per 2 mg tissue, sized per the guide in the preparation step. Agitate the suspension for 20 min at 4 °C. ⏲ 20 min

> ***Critical:*** Tissue samples will take 1–2 h or longer to complete lysis.

(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, pH 8.0 ◇ R0151

(1.) Add room-temperature 1 × urea lysis buffer with inhibitors, 50 µL per $1{\times}10^6$ cells or per 2 mg tissue, sized per the guide in the preparation step. Pipette the sample up and down to break up cell clumps and vortex gently.

> ***Critical:*** Keep the lysate at room temperature throughout. Urea crystallizes at 8 M on ice. Heating above 30 °C carbamylates the proteome (Kollipara and Zahedi, 2013). Do not boil.

(2.) *Optional:* Reduce the viscosity of the lysate by sonication as in the preparation step, in short pulses with the tube returned to a room-temperature water bath between pulses rather than to ice. ⊞

> ***This is why:*** Nucleases are inactive in 8 M urea, so sonication is the only route to shearing the DNA.

(3.) Remove the insoluble fraction by centrifugation at 12 000 × *g* for 20 min at room temperature. ⏲ 20 min

(4.) Collect the supernatant containing the soluble protein fraction in a new tube. Process the same day, or snap-freeze and store at −80 °C; thaw at room temperature and vortex until the urea has redissolved.

🔗 [WZN+09]

Analyses

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

  > ***Critical:*** Dilute urea lysates to below 3 M urea for the bicinchoninic acid (BCA) or below 6 M for the Bradford assay.

- ○ 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.
- ○ *Optional:* Immunostain for the protein of interest 🗎 SOP 0009 if a specific antibody is available. ⊞

Materials

Preparation of cell lysates

Phosphate-buffered saline ⟋ R0037, pH 7.4

Inhibitor cocktail ⟋ R0090, 100 ×

Cell scraper, plastic

Microcentrifuge tube, round-bottom

Sonicator (*optional*)

Protein extraction with RIPA lysis buffer

Radioimmunoprecipitation lysis buffer ⟋ R0148, pH 7.6, Store at 4 °C. Alternatively, store at −20 °C.

Protein extraction with NP-40 lysis buffer

NP-40 / Tris-Triton lysis buffer

- **NP-40 lysis buffer** ⟋ R0149, pH 7.4, Store at 4 °C. Alternatively, store at −20 °C.
- *or* **Tris-Triton buffer** ⟋ R0150, pH 7.4, Store at 4 °C. Alternatively, store at −20 °C.

Proteome extraction

Urea lysis buffer ⟋ R0151, pH 8.0, Store at room temperature. Use on the day it is made and never refrigerate.

Recipes

Radioimmunoprecipitation lysis buffer (RIPA), pH 7.6

Amount	Ingredient		Stock	Final
2.5 mL	Tris hydrochloride (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]	100%	1.0%
250 mg	Sodium deoxycholate	[302-95-4]	414.55 g/mol	0.5%
250 µL	Sodium dodecylsulfate (SDS) ⟋ R0047 Skin irritation (H315); Serious eye damage (H318)		20%	0.1%
100 µL	EDTA, pH 8.0 ⟋ R0017		0.5 M	1 mM
100 µL	Dithiothreitol (DTT) ⟋ R0015 Skin irritation (H315); Serious eye irritation (H319)	□	1 M	2 mM
To 50 mL	Water, reagent-grade			

Filter sterilize. Add reducing agent right before use. Store at 4 °C. Alternatively, store at −20 °C. ***Critical:*** Due to limited solubility, this buffer cannot be prepared as concentrated stock. ***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

No GHS hazards at the indicated concentrations

□ Hold for EHS review before disposal

Date: Sign: R0148

NP-40 lysis buffer, pH 7.4

Amount	Ingredient		Stock	Final
2.5 mL	Tris hydrochloride (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]	100%	1.0%
100 µL	Dithiothreitol (DTT)	□ R0015	1 M	2 mM
	Skin irritation (H315); Serious eye irritation (H319)			
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. Store at 4 °C. Alternatively, store at −20 °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

No GHS hazards at the indicated concentrations

□ Hold for EHS review before disposal

Date: Sign: R0149

Tris-Triton buffer, pH 7.4

Amount	Ingredient		Stock	Final
500 µL	Tris hydrochloride (Tris-Cl), pH 7.4 R0055		1 M	10 mM
1.0 mL	Sodium chloride (NaCl)	R0046	5 M	100 mM
5.0 mL	Triton™ X-100	R0057	10%	1.0%
	Skin irritation (H315); Serious eye damage (H318); Aquatic toxicity (long-term) (H411)			
250 mg	Sodium deoxycholate	[302-95-4]	414.55 g/mol	0.5%
250 µL	Sodium dodecylsulfate (SDS) R0047		20%	0.1%
	Skin irritation (H315); Serious eye damage (H318)			
10 mL	Glycerol	R0022	50%	10%
100 µL	Dithiothreitol (DTT)	□ R0015	1 M	2 mM
	Skin irritation (H315); Serious eye irritation (H319)			
To 50 mL	Water, reagent-grade			

Filter sterilize. Add reducing agent right before use. Store at 4 °C. Alternatively, store at −20 °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

Aquatic toxicity (long-term)

□ Hold for EHS review before disposal

Date: Sign: R0150

Urea lysis buffer, pH 8.0

Amount	Ingredient		Stock	Final
2.5 mL	Tris hydrochloride (Tris-Cl), pH 8.0 R0055		1 M	50 mM
24.0 g	Urea, ultrapure	[57-13-6]	60.06 g/mol	8 M
To 50 mL	Water, reagent-grade			

Dissolve the urea in the Tris-Cl and about 25 mL water at 25–30 °C with stirring, then bring to volume. Store at room temperature. Use on the day it is made and never refrigerate. ***Critical:*** Do not warm above 30 °C: urea decomposes to cyanate, which carbamylates lysines and N-termini irreversibly. ***This is why:*** Tris, as a primary amine, scavenges the cyanate that forms on urea decomposition. 50 mM ammonium bicarbonate serves the same purpose and is volatile in mass spectrometry. For amine-reactive labeling such as TMT, exchange the lysate into triethylammonium bicarbonate after lysis, as in the FASP workflow. HEPES has no primary amine and does not scavenge.

Urea lysis buffer

50 mM Tris-Cl, 8 M Urea, pH 8.0

No GHS hazards at the indicated concentrations

□ Flush to drain with excess water

Date: Sign: R0151

Troubleshooting

Choosing a suitable lysis buffer

In Step 1:

– 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.

Preparation of cell lysates

In Step 2:

– Low protein yield
  - Verify cell count and ensure sufficient lysis buffer volume, at least 1 mL per $1{\times}10^7$ cells.
  - Extend lysis time or add sonication to improve solubilization of membrane and nuclear proteins.
– 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.

List of references

L. Kollipara and R.P. Zahedi, *Proteomics* **13**(6), 941—944 (2013).

J.R. Wiśniewski, A. Zougman, N. Nagaraj, and M. Mann, *Nat. Methods* **6**(5), 359—362 (2009).

Change log

2020-03-22	Shany Koren-Hauer	Initial version.
2025-02-22	Benjamin C. Buchmuller	Adaptation as SOP; scope extended beyond RIPA to NP-40 and Tris-Triton, informed by Invent Biotechnologies "RIPA Buffer: The Potential Troubles" mini-review.
2026-03-03	Benjamin C. Buchmuller	Corrected volumes for final DTT concentration.
2026-05-22	Benjamin C. Buchmuller	Corrected volumes for Tris-Triton buffer Triton X-100 (500 µL → 5.0 mL; v1 prepared at 0.1% vs intended 1.0%, significantly weaker detergent for cytoskeletal extraction) and Glycerol (5.0 mL → 10 mL; v1 prepared at 5% vs intended 10%, mildly less protein stabilization). Same shape as the prior DTT correction.
2026-09-07	Benjamin C. Buchmuller	Added a cell-number and lysis-volume guide. Corrected the tissue-to-buffer ratio (5 mg → 10 to 20 mg per 1 mL; v1 gave about 0.5 mg/mL). Reworked the proteome extraction after Wiśniewski et al., 2009: 8 M urea in 50 mM Tris-Cl pH 8.0 instead of 9 M urea in 200 mM HEPES, boil step removed, all urea steps at room temperature.

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