Whole cell lysates from tissue culture cells

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

This is a bench card. Full protocol available online.

›› *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

›› *Preparation of cell lysates*

(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

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.

Whole cell lysates from tissue culture cells

- 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.

(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*

(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*

(1.) Add ice-cold 1 × NP-40 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.

C › *Proteome extraction*

(1.) Add room-temperature 1 × urea lysis buffer with inhibitors, 50 µL per 1×10^6 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.

(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.

List of references

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

⌀ Recipe (available online) ☼ Troubleshooting (available online) 🕮 Notes (available online)

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