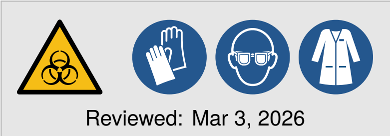
Whole cell lysates from tissue culture cells



Reviewed: Mar 3, 2026

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.

This is a bench card. Full protocol available online.

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	

›› Preparation of cell lysates

☐ Phosphate-buffered saline, pH 7.4	☐ R0090 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.

(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.

A › Protein extraction with RIPA lysis buffer

☐ R0148 Radioimmunoprecipitation lysis buffer, 50 mL

(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

☐ R0149 NP-40 lysis buffer, 50 mL

(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.

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C › ***Proteome extraction***

- [ ] R0151 Urea lysis buffer, 50 mL

(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.

(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.

Recipe (available online) Troubleshooting (available online) Notes (available online)

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