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Lysate Preparation: Organs and Tissue Culture Cells

How one prepares a lysate is crucial, especially when studying phosphorylated proteins. Proteins rapidly dephosphorylate upon harvesting, so timing is critical when preserving phosphorylated proteins. Along with timing, the detergents used in preserving phosphorylation are a key component. When lysing is done properly, a large pool of lysed cells or organs can be prepared and stored in working volumes for long term studies. Preparing pools for studies is an important component to consistent and reproducible results.

PhosphoSolutions' step-by-step protocol shows how to properly lyse organs and tissue culture cells when focusing on preserving protein phosphorylation, including detailed differences in lysing adherent and suspension tissue culture cells. Within each section there is a detailed list of steps, buffers, and specific materials to help researchers through this process. Additionally, helpful technical tips are offered throughout the protocol to provide insight about various factors that should be considered when lysing organs and tissue culture cells. Our main goal is to provide essential techniques or tools to help researchers obtain publishable and reproducible results.

Lysate Preparation Protocols for:

-Organs – Whole Cell Lysate

-Adherent Tissue Culture Cells



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**Have questions?
We are here to help.
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Before You Begin:

- ✓ **Determine protein mass:**
Organs: Weigh fresh or thawed organs. If frozen, brush off frozen debris from organ. If fresh, wash organ with 1X PBS and dry with Kimwipe. Estimate protein mass as 10% of total mass.
TC Cells: Make estimate based on confluency (adherent) and cell count (suspension).
- ✓ **Heat block:** set to 95°C
- ✓ **Sonicator:** Select probe and optimize strength based on lysis buffer volume and tube size to avoid foaming.

Materials Required:

- ✓ **Lysis Buffer:** 1%(w/v) SDS, 10 mM TRIS, 1 mM EDTA, pH 8.0
*room temperature for organs
*95°C for adherent and suspension TC cells
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na₂HPO₄, 5.4 mM KCl, 2.9 mM KH₂PO₄, pH 7.6
- ✓ **Cell Scraper:** For adherent tissue culture cells
- ✓ **Dissection tools, scalpel, spatula, scissors**
- ✓ **Plastic transfer pipette**
- ✓ **Conical tube or microtube with screwcap**
- ✓ **Ice/water**

Prepare Samples

A. Organs- Whole cell lysate

1. Place organ in conical tube or microfuge tube with screw cap. For large organs cut into 1/4" sections. For organs that are thick and dense cut even smaller sections.
2. Add lysis buffer prepared at room temperature. Choose a volume based upon desired concentration, which is based on the estimated mass. Keep sample at room temperature.

Tech Tips:

- a. 1% SDS lyses everything, preserving proteins from protease and phosphatase enzymes. Cocktail inhibitors are not necessary.
- b. A concentration below 10 mg/ml is recommended for optimal lysing. Calculate concentration with formula below:

$$\text{Protein Concentration} = \frac{(\text{Total mass} \times 10\%) \text{ mg}}{(\text{Volume of Lysis Buffer}) \text{ ml}}$$





B. Adherent Tissue Culture Cells

1. Wash cells with 2-10 mls of room temperature 1X PBS once. Remove wash with transfer pipet.
2. Add 95°C lysis buffer, enough to cover the entire plate.

Tech Tip:

- a. Recommended volumes: 2 mls for 150 mm plate, 1 ml for 100 mm plate, 500 ul for 60 mm plate.
3. Rock and rotate the plate to thoroughly coat cells.
Tech Tip:
 - a. Adherent cells will immediately lyse and a glob of cells in lysis buffer will be present.
 4. Transfer to a conical tube or microfuge tube with screwcap.
Tech Tip:
 - a. Tilt plate and gently guide lysed cells with cell scraper to collect at the bottom of the plate. Cut off the tip of a plastic transfer pipette to help collect and transfer the large glob of cells into conical tube.

C. Suspension TC Cells

1. Pipette media and cells into conical tube.
2. Pellet cells by centrifugation (1 minute at 1800 x *g*) and remove media.
3. Resuspend cells with 1-3 mls of room temperature 1X PBS to wash.
4. Repeat step #2.
5. Add 95°C lysis buffer.

Lyse Samples

1. Sonicate sample in 5-20 second intervals until buffer is clear and can be easily pipetted without clogging.

Tech Tips:

For optimal lysing:

- a. To prevent overheating, place conical or microfuge tube in an ice/water bath while sonicating. Do not keep sample in ice bath for an extended period of time.
- b. To avoid puncturing/melting the plastic tube, minimize prolonged contact with the probe to the conical tube walls.
- c. To avoid foaming, keep probe tip in lysis buffer until sonication is complete.
- d. If the lysate sample clogs while pipetting, sonicate again until clog is no longer present.





2. Heat sample at 95°C for 10 minutes. Cool to room temperature.

Tech Tips:

If lysate appears:

- a. Cloudy: Add 5% by volume increments of lysis buffer to sample, then gently rock until sample is clear in solution. A protein concentration below 10 mg/ml is preferred. A concentration above this will appear cloudy and insufficiently lysed.
 - b. "Goopy": Repeat sonication until sample is easily pipetted.
3. Centrifuge lysate at 1800 x *g* for 5 minutes to pellet cell debris.
4. Repeat steps 1-3 if debris is present. Place lysate in fresh conical or microfuge tube. Save/discard old tube and debris.

Tech Tip:

- a. Organ and cell membranes will appear as the white, stringy debris in lysate. It is essential to completely lyse the debris when studying transmembrane proteins.

Determine Protein Concentration

Determine protein concentration using BCA, Bradford, or Lowry assays.

