

Cell Harvest for Protein Assay & Western Blot

Before Starting:

- Turn on heat block to 97.5°C
- Place 1x PBS on ice
- Place TNEB lysis buffer (stored at 4°C) immerse in ice bucket
 - Add fresh Protease Inhibitor Cocktail (Stocks stored at -20°C)
 - 1:1000 Dilution
- Label two tubes
 - 1 for Protein Assay (**without** Laemmle buffer)
 - 1 for Western Blot Sample (**with** Laemmle buffer)
- Prepare western sample buffer (scale as needed)
 - 4.75mL Laemmle buffer (Stock at room temperature)
 - 250µL of BME (Stock in room temperature chemical cabinet)
 - Add 300µL of Laemmle/BME to the tube labeled for Western Blot Sample

Protocol

1. Place tissue culture vessel containing cells on ice.
2. Aspirate culture media and immediately wash twice with ice cold 1x PBS.
3. Remove second 1x PBS wash and immediately add 600µL TNEB.
 - a. **Note:** This volume can change depending on the size of the culture vessel...
4. Scrape lysed cells with rubber policeman.
 - a. **Note:** For a more concentrated protein sample, reuse this cell/TNEB mixture in other wells of the same condition.
 - b. **Note:** Try not to create bubbles.
5. Collect TNEB/Cells via pipette into microcentrifuge tube labeled, “protein assay” for the appropriate condition.
6. Sonicate cells on a setting of 2 (which is equal to between 5 and 6 Watts) for 10 seconds.
7. Vortex the sample.
8. Remove 300µL of the cell suspension and add this to the microcentrifuge tube labeled “Western Blot Sample.”
 - a. The WB sample tube should already contain 300µL of Laemmle buffer / BME
 - i. i.e. this is a 1:1 dilution of cell sample and sample buffer
 1. Total volume = 600µL
 - a. Note: You will use this dilution to correct protein concentration,
9. Heat Western Blot Samples now mixed with Laemmle buffer / BME for 10 minutes on the heat block.
10. Remove Western Blot Samples from heat block and store at -20°C.
11. The remaining 300µL of TNEB/Cells can also be stored at -20°C until you are ready to do the protein assay.

Recipes:

TNEB Protein Lysis Buffer:

| | Volume | |
|---------------------------|----------|----------|
| | 100mL | 200mL |
| MilliQ Water | 98mL | 196mL |
| Tris Base | 605.7mg | 1.2114g |
| EDTA | 74.44mg | 0.149g |
| NaCl | 876.6mg | 1.7532g |
| β -glycerophosphate | 172.8mg | 345.6mg |
| Sodium Orthovanadate | 1.8391mg | 3.6782mg |
| Triton-X 100 | 1mL | 2mL |
| Protease Inhibitor | 1mL | 2mL |

Ordering Information:

| | | |
|----------------------------|--------|----------|
| Tris Base | Sigma | BP152-1 |
| EDTA | Sigma | ED2P |
| NaCl | Sigma | S9625 |
| β -glycerophosphate | Sigma | G-6376 |
| Sodium Orthovanadate | Sigma | 450243 |
| Triton-X 100 | Sigma | X-100 |
| Protease Inhibitor | Sigma | P8340 |
| Laemmle Buffer | BioRad | 161-0737 |
| Beta-Mercaptoethanol (BME) | Sigma | M7154 |