1. Sample lysis

1. **Preparation of lysate from cell culture**
    1. Place the cell culture dish in ice and wash the cells with ice-cold PBS.
    2. Aspirate the PBS, then add ice-cold lysis buffer (100 µl per 1 6 well on 6 well plate) Scrape adherent cells off the dish using a cold plastic cell scraper, then gently transfer the cell suspension into a pre-cooled microcentrifuge tube.
    3. Maintain constant agitation for 30 minutes at 4°C.
    4. Spin at 16,000 x g for 20 minutes in a 4°C pre-cooled centrifuge.
    5. Gently remove the tube from the centrifuge and place on ice. Transfer the supernatant to a fresh tube kept on ice, and discard the pellet.

2. Sample preparation

1. Remove a small volume (50 µl) of lysate to perform a protein assay. Determine the protein concentration for each cell lysate.
2. To reduce and denature: Boil each cell lysate in sample buffer at 100°C for 5 minutes and aliquot. Store lysates at -20°C. Note: aliquot cell lysates (50- 100 µl) to avoid repeat freeze/thaw cycles.
3. Defrost tubes containing cell lysate at 37°C. Centrifuge at 16,000 x g in a microcentrifuge for 5 minutes.

3. Loading and running the gel

1. Load equal amounts of protein into the wells of the SDS-PAGE gel, along with molecular weight markers. Load 20- 30 µg of total protein from cell lysate.
2. Run the gel for 1 to 2 hours at 100 V.

4. Transferring the protein from the gel to the membrane

1. Prepare the transfer stack as follows:

    +

    Sponge

    Filter paper

    Gel

    Membrane

    Filter paper

    Sponge

    -

2. Transfer 2h in 30V.

5. Antibody staining

1. Block the membrane for 1 hour at room temperature or overnight at 4°C using 5% BSA.
2. Incubate membrane with 1:500 dilutions of primary antibody (CD73) in 5% BSA overnight at 4°C or for 2 hours at room temperature.
3. Wash the membrane in three washes of TBST, 5 minutes each.
4. Incubate the membrane with the recommended dilution of labeled secondary antibody in 5% blocking buffer in TBST at room temperature for 1 hour.
5. Wash the membrane in three washes of TBST, 5 minutes each, then rinse in TBS.
6. For signal development, follow the kit manufacturer's recommendations.
7. Remove excess reagent and cover the membrane in transparent plastic wrap.
8. Acquire image using darkroom development techniques for chemiluminesence, or normal image scanning methods for colorimetric detection.