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Cell lysis and immunoblotting

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We use this protocol and it's working

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Abstract

This is cell lysis and immunoblotting protocol.

Troubleshooting

Harvest and Lyse cells

1 Cells were cultured in the presence of the corresponding stress to 60-80% confluency in 6-well plates, 10 cm or 15 cm dishes. After removing the media, the cells were washed with DPBS three times.

2 To lyse cell urea buffer (8M urea, 50 mM TRIS 7.5, 150 mM NaCl, containing mammalian protease inhibitor cocktail (Sigma), Phos-STOP, and 20 unit/ml Benzonase (Millipore)) was added directly onto the cells. Cell lysates were collected by cell scrapers and sonicated on ice for 10 seconds at level 5, and lysates were cleared by centrifugation (15000 rpm, 10 min at 4 ^{O}C).

Run SDS-page gel and transfer to membrane for immunoblotting

3 The concentration of the supernatant was measured by BCA assay. For immunoblotting, the whole cell lysate was denatured by the addition of LDS sample buffer supplemented with 100 mM DTT, followed by boiling at 95^{O}C for 5 minutes

4 10-20 mg of each lysate was loaded onto the 4-20% Tris-Glycine gel (Thermo Fisher Scientific) or 4-12% NuPAGE Bis-Tris gel (Thermo Fisher Scientific), followed by SDS-PAGE with Tris-Glycine SDS running buffer (Thermo Fisher Scientific) or MOPS SDS running buffer (Thermo Fisher Scientific), respectively.

5 *For Chemiluminescence westerns:* The proteins were electro-transferred to PVDF membranes (0.45 µm, Millipore), and then the total protein was stained using Ponceau (Thermo Fisher Scientific). The membrane was then blocked with 5% non-fat milk (r.t., 60 min) incubated with the indicated primary antibodies (4^{O}C, overnight), washed three times with TBST (total 30 min), and further incubated either with HRP conjugated anti-Rabbit and anti-mouse secondaries at (1:5,000) for 1h. After thorough wash with TBST for 30 min membranes were treated with Lightning™ Plus Chemiluminescence Reagent (PerkinElmer, NEL104001EA) after mixing the Enhanced Luminol Reagent and the Oxidizing Reagent 1:1. Mixed Chemiluminescence Reagent was added to blot and rocked gently for 1 minute and imaged using BioRad ChemiDoc Imaging System

6 *For LI-COR westerns:* The proteins were electro-transferred to nitrocellulose membranes and then the total protein was stained using Ponceau (Thermo Fisher

Scientific). The membrane was then blocked with LI-COR blocking buffer at room temperature for 1h. Then membranes were incubated with the indicated primary antibodies (4^{o}C, overnight), washed three times with TBST (total 30 min), and further incubated either with fluorescent IRDye 680RD Goat anti-Mouse IgG H+L, or IRDye 800CW Goat anti-Rabbit IgG H+L secondary antibody at (1:10,000) at room temperature for 1h. After thorough wash with TBST for 30 min, near infrared signal was detected using OdysseyCLx imager and quantified using ImageStudioLite (LI-COR).