Tabas Laboratory Protocol for CHOP Immunoblots of Whole Cell Extracts

Phase1: Cell extract

Remember to culture cells in an abundant amount of fresh media to avoid nutrient depletion-mediated induction of CHOP.

Sample Lysis Buffer (10mls):

2% SDS---------------------------------1.0 ml 20% SDS stock
62.5 mM Tris HCl pH 6.8------------625 µl of 1M Tris HCl pH 6.8
10% glycerol---------------------------1.0 ml glycerol
50 mM DTT----------------------------400 µl of 1.25M stock
.01% Bromphenol blue---------------1 mg
ddH_2O-----------------------------------to 10 ml total volume

1. Aspirate media.
2. Wash cells in ice cold PBS and aspirate.
3. Add 100ul of Sample Lysis Buffer to each well.
4. Scrape cells (the lysate will become viscous).
5. Collect lysate in Eppendorf tube.
6. Boil samples for 5 min.
7. Freeze at -20ºC or load 30 µl on a 4-20% gel.

Phase 2: Western Blot

Primary antibody:
Name: GADD153 (B-3)
Mouse monoclonal IgG_1, 200 µl/mg
Santa Cruz Biotechnology
Cat. # sc-7351 (we have been using Lot # B2603, which is very good)

Secondary antibody:
Peroxidase AffiniPure Donkey Anti-Mouse IgG (H+L)
Jackson ImmunoResearch Laboratories, Inc.
Cat. # 715-035-151

1. If frozen, thaw samples and boil the tubes for 10 min followed by a quick microfuge spin.
2. Add 25 µl of 5X loading buffer into each tube.
3. Load 40 µl of each sample onto lanes of a 4-20% minigel.
4. Run the gel at 100 V.
5. Transfer for 2 hours at 70 V (put whole transfer device into a foam container with ice-water).

6. After transfer, stain protein with Ponceau red to assess quality of gel and equality of protein load per lane.
7. Block in 2.5% non-fat milk/1X TBS-0.1% Tween 20 (TBST) at room temperature for one hour with shaking.
8. Place the membrane in a suitable container and add 1:250 anti-CHOP Ab in 2.5% non-fat milk/ TBST. Cover the box with plastic wrap and incubate overnight in the cold room in a shaker.
9. The next day, quickly wash the membrane 1X with TBST. Then wash 3X with TBST for 10 minutes each.
10. Incubate the membrane for one hour at RT with 1:2000 secondary Ab in 2.5% non-fat milk/ TBST.
11. Quickly wash the membrane 1X with TBST, then wash 3X with TBST for 10 minutes each.

Phase 3: Develop

1. Place the membrane directly from the TBST solution into a suitable container.
2. In a 15-ml conical tube, put 5 ml each of the chemiluminescence developing solution.
3. Pour the solution onto the membrane and swirl for 1 minute.
4. Dry the membrane lightly on Kimwipes.
5. Put membrane face down on plastic wrap, place into X-Ray cassette, and develop.

Results: CHOP gives a clear band at around 29 kDa, and there is a nonspecific band at around 45 kDa.