

Protocol for Mini Western blot using MAb to P-glycoprotein (C219) (Prod. No. ALX-801-002):

Harvesting the MDCK-MDR1 cells:

1. Remove medium from Petri dish.
2. Wash with 4ml of ice-cold PBS (Ca²⁺ and Mg²⁺ free).
3. Add 4ml of 1x Trypsin/EDTA (Cold) and incubate for 5-10 minutes at 37°C. Trypsin is not good for cells and cells should not be over-incubated.
4. While incubating, prepare a sterile 15ml conical tube with 8ml of ice-cold PBS (Ca²⁺ and Mg²⁺ free).
5. When cells look rounded and floating, wash cells from the dish bottom with a cotton-plugged Pasteur pipette. Resuspend well.
6. Transfer the cell suspension to the centrifuge tube with prepared with ice-cold PBS (Ca²⁺ and Mg²⁺ free).
7. Centrifuge the cells at 400 x g for 10 minutes at 4°C.
8. Remove PBS and resuspend in 1ml of fresh ice-cold PBS (Ca²⁺ and Mg²⁺ free).
9. Transfer to a sterile microcentrifuge tube.
10. Centrifuge the cells at 400 x g for 10 minutes at 4°C.
11. Remove PBS and immediately put into liquid nitrogen to quickly freeze the cell pellet.
12. Store at -80°C.

Protein Assay:

For 250ml of Laemmli buffer use: 1.52g TRIS-Cl, pH6.8 (50mM)
 50ml 10% SDS (2% SDS final concentration)
 25ml glycerol (10% glycerol final concentration)

To 10ml of Laemmli buffer add: 50µl of 1M DTT
 5µl of 1m PMSF
 1 complete mini protease inhibitor tablet
 ***This solution must be used within 10 minutes!**

1. Remove cells from the -80°C and resuspend with Laemmli buffer with supplements.
2. Sonicate 2x for 1 minute on ice.
3. Perform two freeze/thaw cycles on the cells. First, put cells in liquid nitrogen to freeze, then in a 37°C water bath until thawed; repeat.
4. Put cell suspension in an insulin syringe and push needle 10x.
5. Measure protein concentrations.

Western blot:

Sample Preparation:

1. Load 12.5µg cell lysate per lane and electrophorese on a 4-20% gradient gel.
2. Transfer to a polyvinylidene difluoride membrane, 1 mini gel in a wet transfer for 1 hour at 150V.
3. Block in 5% non-fat milk PBS-Blotto for a hour at room temperature.
4. Incubate with Monoclonal Antibody to P-glycoprotein (C219) (Prod. No. ALX-801-002) 1:50 in 1% milk for 3 hours on a rocker at 4°C (the low % of milk allows the antibody to be used at least three times if stored at 4°C).
5. Wash 3x for 10 minutes in PBS-Blotto at room temperature.
6. Incubate with the secondary antibody (HPR-conjugated goat anti-mouse) 1:10'000 in 5% milk PBS-Blotto for 1 hour at room temperature.
7. Wash 3x for 20 minutes in PBS-Blotto at room temperature.

The procedures listed above are intended only as a guide. Various assay conditions require that the investigator determine the optimal working concentrations. The results may vary depending on experimental conditions and technique. No warranty or guarantee of performance of above procedure is made or implied. Use good laboratory practices and handle all materials with care.

These products and procedures are for in vitro experimental use only and are not intended for use in humans or clinical diagnosis.