PROTOCOL

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 (Ca^{2+} and Mg^{2+} 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, pepare a sterile 15ml conical tube with 8ml of ice-cold PBS (Ca^{2+} and Mg^{2+} 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 (Ca^{2+} and Mg^{2+} free).
7. Cetrifuge the cells at 400 x g for 10 minutes at 4°C.
8. Remove PBS and resuspend in 1ml of fresh ice-cold PBS (Ca^{2+} and Mg^{2+} free).
9. Transfer to a sterile microcentrifuge tube.
10. Cetrifuge 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 sryinge 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 diflouride 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.