PHOTOCONVERSION OF ANTIBODY LABELED CULTURED CELLS

Description: This protocol is for EM level immunolocalization of proteins by photoconversion using eosin.

Cells are grown on MatTek dishes.

1) Fix in 4% formaldehyde (made fresh from paraformaldehyde) in 0.1M PBS for 20 minutes (first 5 minutes at 35°C then on ice). If your antigen can tolerate low concentrations of glutaraldehyde (i.e. 0.1-0.5%), then include this in the fixative.

2) Wash in PBS then PBS with 0.05M glycine plus an appropriate amount of detergent (i.e. 0.1% triton X-100 or saponin) for 10 minutes.

3) Block in 2% normal goat serum and 1% cold water fish gelatin (Sigma) in 0.1M PBS-glycine for 20 minutes.

4) Wash for 5 minutes in working buffer (0.1% NGS and 0.1% CWFG in 0.1M PBS).

5) Into primary antibodies for 1-2 hour @ 4°C:

6) Wash in working buffer 6 x 5 minutes.

7) Into secondary antibody (i.e. goat anti-rabbit IgG eosin diluted 1:50 for 1 hour).

8) Wash in working buffer 6 x 5 minutes.

9) Observe with confocal microscopy. A dish should be done in parallel using an FITC conjugated secondary. The eosin fluorescence will be about 20% as bright as FITC. In order for successful photoconversion the fluorescence signal should be strong.

10) Wash with CSB 4 x 4 minutes and observe with confocal

11) Fix with 2% glutaraldehyde for 10 minutes in CSB on ice.

12) Wash with 0.1M sodium cacodylate 4 x 2 minutes.

13) Add 0.5mg/ml DAB in 0.1M sodium cacodylate and photooxidize.

14) Wash with 0.1M sodium cacodylate 4 x 2 minutes.

15) Post-fix with 1% OsO_4 in 0.1M sodium cacodylate for 30 minutes.

16) Rinse in DDW

17) Dehydrate in ETOH series.

18) Infiltrate with 50:50 Durcupan-ETOH for 30 minutes

19) Infiltrate with 100% resin 1 hour x2.

20) Polymerize at 60°C for 24 hours.