^{125}I- ANTI-Ig INDIRECT ASSAY FOR MONOCLONAL ANTIBODIES BINDING TO CELL SURFACE ANTIGENS

Monoclonal antibodies are incubated with target cells, and active binders are detected with a second ^{125}I-anti-Ig reagent with the appropriate species specificity. Usually the MAb is in excess, and either the number of target cell antigen sites or the ^{125}I-antibody are limiting in this assay. When testing clonal supernatants, dilutions of 1/10, 1/100, and possibly 1/1000 should be used, since it is important to choose totally active clones rather than clones which have become mostly inactive through chain loss but which might appear to give the same binding if used undiluted.

1. Put 10 µl of hybridoma culture supernatant into each well of a microtiter V-well polystyrene 'test' plate using an adjustable pipette and changing or rinsing tips between each supernatant. Supernatants which will be tested in a number of assays are conveniently stored in 'master' plates, 50-200 µl/well, and transferred with a 12 tip pipettor to 'test' plates. Include controls of non-binding antibody, standard high-binding antibody, and total input counts.

2. Put 5 µl of specified target cells into each well using a 250 µl Hamilton syringe with repeating dispenser. Usually cells are glutaraldehyde-fixed, stored frozen in 10% BSA at -80°C and used at a concentration of 5 x 10^7/ml. It is essential that the drop of cells and drop of antibody touch, otherwise mixing will not occur even after shaking.

3. Place clear adherent tape over each plate and place them on shaker in cold room. Shake (setting of 8) for 45 min. at 4°C.

4. Remove from shaker and wash 2x. Each wash cycle consists of adding 180 µl of ice cold 0.25% BSA in tris saline/well using Cornwall syringe set at 2 ml with 12 needle manifold, spinning at 300 x g for 5 minutes and aspirating the supernatant using the 12 needle aspiration and guiding the needles down the sides of the wells. At first wash, add 5 µl of 1% glutaraldehyde-fixed human RBC to help prevent washing losses and act as marker for cell pellet. Alternatively, the RBC may be diluted 1:40 into the 0.25% BSA and 180 µl added in the first wash only.

5. After aspirating the second wash, add 5 µl of ^{125}I-labeled antibody with a Hamilton syringe to each well (place the tip of the syringe at the corner between bottom and side of well). Place two aliquots of ^{125}I into counting tubes for total input counts. Shake for 45 minutes at 4°C at top speed. Check after 15 min for complete resuspension by viewing plate from bottom. This is essential for accurate results.

6. Wash 3x.

7. Resuspend cells with 1.5 ml/12 wells. Transfer liquid into test tubes using multichannel pipet set at 150 µl. Wash 1 x by transferring 150 µl of rinse into wells with pipette and also transferring to tubes before proceeding to next row.

Seal tubes with 90 µl of 40:60 w/w paraffin:mineral oil. Warm on hotplate at setting of No. 5. Use pipette tips with 1/16'' cutoff and be careful not to get paraffin into pipette; don't use second stop.

9. Count each sample 0.5 to 2 min. on gamma counter. Place total input counts and empty tubes for background at the beginning. The appropriate sequence for analysis of data with the 'iso' computer program is, for each ^{125}I-antibody/cell type combination: input counts or maximal counts, control with irrelevant MAb, and experimental MAb(s). Make punched tape for computer data entry.