T. Springer, July 1982

HIGH SPECIFIC ACTIVITY ^{3}H-LABELLED Ig FOR CROSS INHIBITION EXPERIMENTS

1. Count a trypan-blue stained suspension of the cells. Wash 10^6 viable cells twice in leucine-free medium at 200 x g for 5 min. at 10°C. The Leu-free medium should contain Gln, antibiotic, and 10% dialyzed FCS. Aspirate using a pasteur pipette with a constricted tip taking care not to remove the pelleted cell mass.

2. During washing, prepare the following amount of [^{3}H] leucine/sample as follows: (do not handle radioactive compounds in forward-flow laminar hoods).

Add in sterile hood: 11 µl 10 x Earle's balanced salts
5 µl FCS
2.5 µl 7.5% $NaHCO_3$

Add to above after transferring to regular bench:
100 µl 1 mCi/ml [^{3}H]L-leucine, NET-460, 110 mCi/mmol, in 0.01 N HCL.

Adjust pH by vortexing and opening to atmosphere as necessary to release CO_2. Store capped until cells are added.

3. Suspend cells in 100 µl of leucine-free medium + Gln, antibiotics, and dialyzed 10% FCS, add to well containing [^{3}H]-leucine prepared in step 2, and mix.

4. Incubate for 6 to 24 h, 37°C, appropriate CO_2.

5. Add 800 µl of 1% BSA in tris-saline-azide (TSA), centrifuge (1,000 x g, 5 min.), remove supernatant, and dialyze vs. TSA O/N. Count a 1 µl aliquot and store at -35 to -80°C.

T. Springer, July 1982

[3H] Ig CROSS-INHIBITION ASSAYS

General: Antibodies recognizing identical or proximal cell surface determinants will inhibit each other's binding. Fresh cells are used in this assay, and it is important to keep cells, microtiter plates, and wash solution on ice, and perform incubations and centrifugations at 4°.

1. Five-fold serially dilute inhibitors (antisera and monoclonal supernatants) in 1% BSA in Hanks-HEPES. Place 50 µl aliquots in microtiter wells.

2. Add 5 µl of cells at concentration giving ~ 75% maximal ^{3}H Ig binding, determined by prior titration, seal with tape, and shake 30 minutes at 4°.

3. Add 5 µl of dialyzed [^{3}H] Ig diluted to 1,000 cpm/µl , reseal, and shake a further 30 min. at 4°.

4. Add 100 µl/well 0.25% BSA Hanks-HEPES, centrifuge at 200 x g for 5 min., and aspirate in <u>center</u> of well to a stop-point determined by maximal upward adjustment of the fine 'focus' on the 12 needle aspirator. Repeat 4x more using 150 µl 0.25% BSA Hanks-HEPES.

5. Suspend cells in 150 µl of Tris-saline, transfer to NEN mini-vials, add 2 drops 1% SDS, thoroughly vortex, add 4 ml Biofluor (NEN), vortex thoroughly 2x with a wait in-between, and count β scintillations.

6. Controls: input cpm, no inhibition (1% BSA), β counter background.