

V79 COLONY FORMING ASSAY

Experiment Name : ³HTdR toxicity (cluster, 100% labeling)

Exp. #: 6

Investigator: R. Howell

Date: 9/27/01

Serum/Lot #s: MEMA 10% Hyclone FCS Lot# AJD10234

MEMB 10% Gibco FCS Lot# 1023298

1. Set the rocker-roller at 37°C incubator with 5% CO₂, set the Coulter Counter, wash cells (from one 225 cm² flasks, subcultured 1:2 the day before) with PBS-PS, trypsinize cells with 2 ml trypsin 3 min at 37°C, resuspend in 10 ml MEMB, pool, pass five times through 10 cc syringe with 21 gauge needle, perform cell count by transferring 100 ul in Coulter cup containing 20 ml Isotone II (Coulter balanced electrolyte solution).

2. Dilute to ~4,000,000 cells/ml in MEMB [Actual count : 3.76×10^6 cells/ml)

500ul Bkg 13 3875 3946 3809 (100ul mean) 1

$$3877 \times 400 \times 5 = 7.75 \times 10^6 / \text{ml} \quad \frac{40 \times 10^6}{7.75 \times 10^6 / \text{ml}} = 5.16 \text{ ml} + 5.84 \text{ ml MEMB}$$

3. Transfer 1 ml of cell suspension into 10 14 ml tubes (Falcon polypropylene test tube, 17x100 mm) labeled 1-10 both on cap and wall.

4. Keep the tubes in the roller for 3-4 h at 37°C, 5% CO₂

Date/Time: 4:20 pm

5. Prepare MEMB containing radioactivity in hood

270 µl ³HTdR (Stock : 1 µCi/µl on 9/19/01) + 4.23 ml MEMB

Manufacturer: Perkin Elmer Lot #: 3106-445 Calibration: 9/19/01
NET-027Z

6. After 3-4 h, remove tubes from roller and add MEMB with radioactivity according to Table below.

After dilution 11202 11202 11078

500ul mean $11160 \times 400 = 4.46 \times 10^6 / \text{ml}$

Add 1ml MEMB 9363 9222 9431
 $9339 \times 400 = 3.74 \times 10^6 / \text{ml}$