CYTOTOXICITY ASSAY - Cr^{51} RELEASE

Materials:

1. MEM + 5% FCS or Medium 199 + 5% FCS (GIBCO # E-12)

2. $Na_2 Cr^{51} O_4$ 5mC/ml in saline from New England Nuclear, 575 Albany St., Boston, Mass. NEZ 030 $35.00

3. Complement - Low toxicity guinea pic C^1 (Hyland Lab). Expect 33% loss of C^1 volume.

   Absorb with agarose. 80 mg agarose/1 ml neat C^1 (or 1:3 in MEM). Incubate at 4°C for 2-5 hours. Then dilute to final conc before spinning and removing C^1.

   Store in -70°C freezer.

   Final C^1 concentration to be determined in assay. ≈1:10 final conc.

Labelling Cells:

1. Incubate cells @ 10^7 - 10^8/ml with 100 μC of $Na_2Cr^{51}O_4$/ml cells for 1 hour @ 37° in humid incubator. This labels sufficiently to detect killing with 5-10 minute counts.

   Increase conc of Cr^{51} to increase labelling without decreasing viability.

   Use 2X number of cells needed if it is a large assay as cells will be lost during washes, etc. 25-30% loss during labelling.

2. Dilute antiserum in titre plates. Dilute more volume than is needed to compensate for Eppendorf accuracy.

3. Wash labelled cells 3X with MEM + FCS (liquid radioactive waste). Gently resuspend each time on vortex speed 3. Centrifuge @ 1200 - 1800 rpm. 5 minutes @ 4°C.

4. Count cells by resuspending in 1 ml and do coulter count. Adjust volume to 1 x 10^6 cells/50 λ or 20 x 10^6/ml.

5. Add 50 λ cells to each tube containing 10 λ of the appropriate antiserum dilution. Shake tubes and incubate at 37° humid incubator for 1 hour.

6. Centrifuge @ < 1500 rpm for 10 minutes. Decant supernatant and blot inverted tube to remove as much moisture as possible (can use drawn out pipet.)

7. Add medium and 50 λ complement to 100λ total volume. Mix each tube well.

8. Incubate 45 minutes to 1 hour @ 37°C.

9. Centrifuge @ 2000 rpm for 5-10 minutes all tubes except freeze-thaw controls (F-T).

10. The FT tubes should be frozen in Ethanol and dry ice and thawed under hot tap water 4 times. Then centrifuged as above.

11. Sample 20 λ of supernatant for each tube using an eppendorf pipet whose tip has been cut in half to fit inside 16 x 125 mm tubes (or use compupet) washing tip between tubes. Cap tubes with critoseal and insert in 13 x 100 lipped counting tubes.

12. Count in gamma counter (change settings for Cr^{51}) for 5-10 minutes.

NOTES:

1. Controls

   Positive Controls:
   a. known positive antiserum
   b. Freeze-Thaw gives total radioactive release possible.

   Negative Controls:
   a. Complement step only. No antiserum in 1st step.
   b. Spontaneous Release - no antiserum or C^1.
   c. Each Antiserum Only - no C^1.

2. Do duplicates or triplicates of each test item.
   Wear gloves to avoid radioactive contact.

3. When adding Ab, proceed from the most dilute to least dilute, wash eppendorf carefully between

   total incubation volume 100λ (add media to reactants to reach 100 λ)

   1st step 50 λ of cells @ 10^6/50λ
   10 λ of antiserum @ 10X final conc.

   2nd step 50 λ of C^1 @ 2X final conc.

4. Complement must always be kept cold until use.

   Horse serum has much less natural cytotoxicity than guinea pig serum to mouse thymus. Weissman's lab uses horse serum @ 1:8.