

ANTI-THETA TREATMENT OF CELLS

2-Step Kill

Purpose of Treatment: Anti-theta serum in the presence of C' has a cytotoxic effect on the T cell portion of a spleen cell population thereby enhancing the percentage of B cells in the population.

It is to be expected that anti-theta treatment will kill 50% of spleen cells.

Preparation of Complement: Hyland Guinea Pig Complement (lyophilized) is reconstituted with 10 ml distilled water (not diluent provided).

The final concentration of C' used in the incubation mixture is 1:10.

To prepare C', allow 3X amount of absorbed C' needed in the final reaction mixture to react with agarose (80 mg agarose/1 ml C'). Mix well and allow to stand 1-3 hrs. in ice. Centrifuge and keep supernate in ice until use. The complement must be absorbed to remove mouse specific cytotoxicity.

Antiserum:

- Treatment:
1. Bring cells to almost 10^7 /ml allowing for addition of other reagents in ISOMEM (no FCS).
 2. Add antiserum or normal serum to make appropriate final serum dilution of _____. Mix well. Place in 37°C water incubator for approx. 30 min. (for 15 ml conical) or 45 min (for 40 ml conical)
 3. Spin down (290 x g approx. 1100 rpm for IEC cent.); resuspend in absorbed C', to final C' dilution of 1:10. Mix well. 1×10^7 cells/ml.
 4. Incubate at 37°C 30-45 min. in covered conical centrifuge tubes.
 5. Centrifuge at 290 x g for 10 min. Wash by resuspending in large volume MEM + 5% FCS.
 6. Centrifuge and resuspend.
 7. Count and adjust volume for injecting.

For Example:

If 5×10^7 cells are needed for injection treat 10×10^7 spleen cells (2X number of cells needed post-treatment).

The final volume of reaction mixture will be 10 ml since final conc. of cells should be 10^7 /ml.

Reaction mixture per 10^8 cells:

1st step

9.75 ml ISOMEM containing cells
0.25 ml antiserum if 1/40
10.00 ml total volume

2nd step

pelleted cells
+ 10.00 ml C' (1/10 dil.)
10.00 ml total volume