

Laboratory Protocols - Ruminant Immunology

Thawing Hybridomas:

Cells must be thawed gently or they will die. As you may imagine, they aren't particularly happy after being stored in liquid nitrogen for months. Also, when frozen (see below) the media contains an organic chemical called Dimethylsulfoxide (DMSO) which prevents ice crystal formation, but inhibits growth. The formula for thawing media is given below.

Materials:

A bottle of incomplete IMDM media, cold, to be used for washing.

A 24-well culture plate

A 15 ml centrifuge tube for each cell line to be thawed.

A 50 ml tube of Complete IMDM (containing Pen-Strep, 2-ME, and Minimal Amino Acids) + 10% FCS + 1% IL-6 supernatant (from the stock in the refrigerator)

-to a 50 ml tube, add 45 ml complete IMDM

5 ml Fetal Calf Serum

0.5 ml IL-6 media (from the stock)

-always warm to 37C in the water bath prior to use.

Methods:

1. Use incomplete IMDM (no supplements) as washing media. Add 10 ml of washing IMDM media to a 15-ml centrifuge tube. Keep sterile. You will need 1 tube for each line.
2. Quickly and safely find vial of frozen cells in liquid nitrogen. NEVER attempt to thaw more than 2 cell lines/person at a time, as the cells will die. Remove vial, place in rack, and return remaining cells to liquid nitrogen.
3. Thaw tube by running under hot water until only a pea-sized ice pellet is visible. Wipe tube with Kleenex and write name of cell line and isotype on the prepared 15ml centrifuge tube.
4. Using a 5 ml pipette, collect ALL of the media and cells in the freezing vial and transfer to the centrifuge tube. Keep vial.
5. Spin the cells at 1500 rpm for 7 minutes. Remove supernatant using vacuum. In order to keep the cells sterile, use a sterile yellow pipette tip attached to a Pasteur pipette.
6. Gently resuspend the cells in 10 ml of washing IMDM.
7. Spin the cells at 1500 rpm for 7 minutes at 4C. Remove supernatant using vacuum, keeping sterile as before.
8. Gently resuspend the cells in 10ml of washing IMDM.
9. Spin the cells at 1500 rpm for 7 minutes at 4C.

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10. While the cells are spinning, prepare a 24-well culture plate as follows. You will use one row for EACH cell line that you are working with.
11. Into Wells #1-6, place 1 ml of Complete IMDM containing 10% FCS and 1% IL-6 supernatant.
12. Collect the tube containing the cells from the centrifuge. Remove supernatant using vacuum, keeping sterile as before.
13. Gently resuspend cells in 2 ml Complete IMDM containing 10% FCS and 1% IL-6 supernatant.
14. Collect all 2 ml of cells using a 5 ml pipette, and add 1 ml of cells to well #1, and 1 ml of cells to well #2.
15. Mix gently up and down a few times, and transfer 1 ml of cells from well #2 to well #3.
16. Mix gently up and down a few times, and transfer 1 ml of cells from well #3 to well #4.
17. Mix gently up and down a few times, and transfer 1 ml of cells from well #4 to well #5.
18. Mix gently up and down a few times, and transfer 1 ml of cells from well #5 to well #6.
19. Self check. Make sure that you have 2 ml in wells #1 and #6, and 1 ml in wells #2,3,4,and 5.
20. Add 1ml of Complete IMDM containing 10% FCS and 1% IL-6 supernatant.
21. Place plate in the incubator. Observe daily for growth of cells (normally takes at least 3 days to get going).