

Laboratory Protocols - Ruminant Immunology

Preparation of IL-6 Rich Media:

When thawing cells, hybridomas initially need the cytokine IL-6 in order to grow. Once growing effectively they no longer require it. IL-6 can be produced in the lab by growing a cell line which has been engineered to produce it, m63X-IL-6. This is grown in standard tissue culture media including a selectable drug, Genatamycin. The formula for media is given below.

Gentamycin/Genticin - G418 Sulphate, Cat#066-1811

Materials:

15-ml centrifuge tube

Incomplete IMDM (no supplements) for washing

50 ml of Genticin-medium, warmed to 37C

-To a 50 ml centrifuge tube, add

45 ml <u>Complete IMDM</u>
5 ml FCS
1 ml (50 mg) Genticin (G418)

Complete IMDM, 10% FCS., warmed to 37C

-To a 50ml centrifuge tube, add

45 ml <u>Complete IMDM</u>
5 ml FCS.

Complete IMDM, no FCS, warmed to 37C

25cm² flask
2-75cm² flasks
1-175cm² flask

Methods:

1. Thaw cells from froze stock as normal. Wash 3 times in 10ml IMDM no supplements.
2. Resuspend in 5 ml IMDM/Genticin medium and add to a 25cm² flask.
3. When growing well, split into 2-75cm² flasks in a total volume of 30 ml Genticin (add 2 ml of culture from 25cm² flask to 28 ml of Genticin IMDM media.
4. Freeze down 5 vials of cells in 90% FCS/10% DMSO as usual.
5. Take 5 ml of cells into 45 ml complete IMDM/10% FCS NO Genticin
6. When growing well, place these 50 ml of cells into 250 ml Complete IMDM NO FCS. Grow until death.
7. Spin down entire culture in 50 ml centrifuge tubes, filter supernatant and throw away pellet, and store at 4C.

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