

PREPARATION OF CULTURE MEDIA:

Complete Medium

500 ml	Iscove's Modified Dulbecco's Medium (IMDM) with L-glutamine and HEPES
100 ml	Fetal Bovine Serum
5 ml	L-glutamine 200mM
5 ml	Sodium Pyruvate 100mM
0.5 ml	Gentamicin 50 mg/ml
0.5 ml	Penicillin-Streptomycin 10,000U/ml Pen and 10mg/ml Strep

Recommended Handling of Cells to Initiate Culture:

Thaw the cells by rapid agitation in a 37°C water bath (thawing should be rapid – within 2 minutes). Decontaminate the outside of the vial by spraying it with 70% ethanol. Inside a laminar flow tissue culture hood, aseptically transfer the cell suspension to 5.0 ml of complete IMDM culture medium in a centrifuge tube. Spin the cells at 170xg for 5 minutes. Carefully draw off and discard the medium containing the freezing additive. Resuspend the cells in approximately 1.0 ml of medium and transfer to a T-75 flask containing 9 ml of complete IMDM culture medium.

Maintenance of Cells in Culture:

Hybridoma cultures are a mixture of suspended and adhered cells and double in number approximately every 24-48 hours. It is recommended to maintain cell density between 5×10^5 and 1×10^6 cells/ml.