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Thawing Hybridoma Cells

Day 1:

1) take cryovial from liquid nitrogen storage and quickly place on dry ice

2) pipet 1 ml of media pre-warmed to 37°C into a 10 cm Petri dish

3) immediately place cryovial in 37°C water bath and thaw watching closely (about 30-60 sec, just until cells are mostly thawed)

4) pipet the cells into the Petri dish containing 1 ml media

5) wait about 20-30 sec, then add 2 ml of media

6) wait about 20-30 sec, then add another 2 ml media

7) wait about 20-30 sec, then add another 2 ml media

8) add 8 ml media and place in 37°C (5% CO_2) incubator

Day 2:

Check cells under microscope to make sure they look healthy (cells should be round, bright, and clear)

If cells look healthy and are sufficiently confluent (50% confluent or greater), split 1:3 into new Petri dishes with media.

If most cells do not look healthy, harvest cells and centrifuge at 500 x g, aspirate media, resuspend in 15 ml of fresh pre-warmed media and replate into a fresh 10 cm Petri dish.

Day 3:

Make sure cells are healthy and split 1:3 into new Petri dishes with media

Media:
10% FetalClone I (ThermoFisher Cat# SH3008003)
1X PenStrep (ThermoFisher Cat# 15140122)
1X NEAA (ThermoFisher Cat# 11140050)
In DMEM (ThermoFisher Cat# 11965092)

Note that there are many suitable alternative protocols (*e.g.*, Growing Hybridomas, Chapter 8. Antibodies: A Laboratory Manual. Second Edition. Greenfield, E. A. 2014. Cold Spring Harbor Laboratory Press.)