SOP: **Propagation of Mouse 416B cells**
Date modified: **01/12/2011**
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Ordering Information

Mouse 416B cells were received from Dr. Marella deBruijn, The Weatherall Institute of Molecular Medicine, Oxford University, Oxford, England.

Notes:
This is a mouse hematopoietic suspension cell line.

Materials List

1. MEM Alpha Medium, 1X, with L-Glutamine, without ribonucleosides and deoxyribonucleosides (Gibco/Invitrogen, Cat# A10490-01)
2. Horse Serum (Gibco/Invitrogen, Cat# 16050-122)
3. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
4. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
5. T75, T225 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated serological pipets (1, 5, 10, 25, 50mL)
8. 2X Freezing Medium (80% horse serum + 20% DMSO)
9. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
10. CryoVials (Nunc, Cat# 368632)
11. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
12. Eppendorf Centrifuge 5810R
13. Revco UltimaII -80°C Freezer
14. Thermolyne Locator 4 Liquid Nitrogen Freezer
15. Hemocytometer
16. Micropipet w/ P20 tips
17. Microscope

Growth Medium for Mouse 416B Cells

MEM Alpha Medium, 1X, with L-Glutamine, without ribonucleosides and deoxyribonucleosides
20% Horse Serum
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Cultures

1. Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2. When ready to start cell culture, quickly thaw ampoule in 37°C water bath.
3. As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol.
4. Pipet thawed cells slowly into a 15mL conical centrifuge tube containing 1mL 100% horse serum (equal volume to the cells in cryovial).
5. Add 10mL complete growth medium to dilute out the DMSO in the freezing medium.
6. Centrifuge cells for 5 minutes at 200 x g (4°C).
7. Discard supernatant and tap to loosen the pellet.
8. Starter culture should be set up at $3x10^5$cells/mL. Add growth medium to achieve this concentration and place in a T75 flask.
9. Place flask in a 37°C, 5% CO_2 humidified incubator.

B. Sub-culture and Maintenance

1. Check cell count with a hemocytometer every 18-20 hour. Count cells in the T75 starter culture. Seed cells at $2x10^5$cells/mL concentration in a T225 flask.
2. Maintain cell density between $2x10^5$cells/mL and $8x10^5$cells/mL by adding fresh medium. Continue to dilute cells in this manner until one has a sufficient number of cells for seed stock freeze down and storage, and experimentation.
3. **Concentration of cells should never exceed $8x10^5$cells/mL**. Cultures will reach this concentration if left growing for longer than 20 hours.
4. Record each subculture event as a passage.

C. Freezing Down Seed Stock Cells

1. At an early stage of expansion and with sufficient number of cells to continue maintenance, a small portion of the cells should be set aside as a seed stock, if needed.
2. Make 2X freezing medium (80% horse serum + 20% DMSO) and place on ice.
3. Label cryovials with name of cell line, cell number, date, and preparer's initials. Place these cryovials on ice.
4. Count cells designated for seed stock and place in a conical centrifuge tube. Centrifuge at 500 x g (4°C) for 5 minutes.
5. Aspirate supernatant and resuspend the cell pellet in 1X PBS to wash. Centrifuge again under same conditions.
6. Resuspend cell pellet in cold growth medium at $2x10^7$cells/mL.
7. Add equal volume of 2X freezing medium to cells and invert tube to mix gently. Place on ice.
8. Pipet 1 mL of cells (now in 1X freezing medium) per cryovial (10^7 cells).
9. Place cryovials in a Nalgene Cryo 1°C freezing container.
10. Leave cells overnight in a -80°C freezer, then transfer to permanent liquid nitrogen storage.

D. Harvest

1. Passage cells until the desired number of cells for experimentation is reached in a logarithmic growth phase.
2. Pellet cells and rinse with 1X PBS as described in "Freezing Down Seed Stock Cells" section.
3. Examine viability using Trypan blue staining (SOP TP-7).