SOP: Propagation of Mouse 416B cells

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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% CO₂ 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 2x10⁵cells/mL concentration in a T225 flask.
- 2. Maintain cell density between 2x10⁵ cells/mL and 8x10⁵ 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 8x10⁵ 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 (10⁷ 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).