SOP:	**Propagation of Mouse A20 Lymphoblasts**
Date modified:	**01/18/2011**
Modified by:	**T. Canfield (UW)**

Ordering Information

Mouse A20 Lymphoblasts can be ordered from ATCC as a frozen ampoule. This is a mouse suspension lymphoblast cell line derived from a reticulum cell sarcoma (B cell lymphoma).

Name: A20—Mouse Reticulum Cell Sarcoma Lymphoblast
ATCC #: TIB-208

Materials List

1. RPMI 1640 with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate, 4.5g/L glucose, 10mM HEPES, and 1.0mM sodium pyruvate Medium (ATCC, Cat# 30-2001)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. T75, T225 tissue culture flasks
4. Corning conical centrifuge tubes (15mL and 50mL)
5. Graduated pipets (1, 5, 10, 25, 50mL)
6. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
7. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
8. 2-Mercaptoethanol, liquid, cell culture tested (Sigma-Aldrich, Cat# M7522)
9. Freezing Medium (growth medium containing 5% DMSO)
10. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
11. Cryovials (Nunc, Cat# 368632)
12. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
13. Eppendorf Centrifuge 5810R
14. Revco UltimaII -80°C Freezer
15. Thermolyne Locator 4 Liquid Nitrogen Freezer
16. Hemocytometer
17. Micropipet w/ P20 tips
18. Microscope

Growth Medium for Mouse A20 Lymphoblasts

RPMI 1640 with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate, 4.5g/L glucose, 10mM HEPES, and 1.0mM sodium pyruvate Medium
10% Characterized Fetal Bovine Serum
0.05mM 2-Mercaptoethanol
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2) When ready to start cell culture, quickly thaw ampoule in a 37°C water bath.
3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, and then transfer the vial contents to a 15mL conical centrifuge tube containing 9mL of complete growth medium.
4) Centrifuge at 125 x g (4°C) for 7 minutes.
5) Resuspend the cell pellet in complete pre-warmed growth medium at a cell concentration of $2x10^5$cells/mL in a T75 flask and grow in a 37°C, 5% CO_2 humidified incubator.

B. Sub-culture and Maintenance

1. Take cell counts with a hemocytometer every 24 hours to maintain the culture at a cell density between $1x10^5$cells/mL and $1x10^6$cells/mL. The cells have a doubling time of 18 hours and **the concentration of cells should not exceed $1x10^6$cells/mL.**
2. Add fresh warm medium when appropriate to maintain cell density and expand the culture to the desired number of cells.
3. Record each subculture event as a passage.

C. Generation of Seed Stocks

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. Amount of cells for the seed stock should be placed in a conical centrifuge tube and centrifuged at 500 x g (4°C) for 5 minutes.
3. Aspirate supernatant and resuspend the cell pellet in 1X PBS to wash. Centrifuge again under same conditions.
4. Resuspend the cell pellet in freezing medium (growth medium containing 5% DMSO) at a concentration yielding 2 million cells per 1mL aliquot.
5. Dispense 1mL cell suspension per cryovial. Place cryovials in a Nalgene Cryo 1°C freezing container and store overnight at -80°C.
6. Cryovials are transferred the next day to liquid nitrogen freezer for long-term 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 in "Generation of Seed Stocks" section.
3. Examine viability using Trypan blue staining (SOP TP-7).