MAINTENANCE OF M2-10B4 AND Sl/Sl CELL LINES

DESCRIPTION:

M2-10B4
M2-10B4 is a stromal cell line derived from the bone marrow of a (C57BL/6J x C3H/HeJ) F1 mouse.[1] This cell line supports human myelopoiesis in long term culture (LTC).[2,3]
Available from American Tissue Culture Corporation ATCC number: CRL-1972.

M2-10B4 (IL-3, G-CSF) Cell Line **Code 00301**
M2-10B4 stromal cell line has been genetically engineered to produce human interleukin-3 (IL-3) and human granulocyte colony stimulating factor (G-CSF). This cell line supports human myelopoiesis in LTC initiating cell (LTC-IC) assay. [3]

Sl/Sl (IL-3, SCF) Cell Line **Code 00302**
Sl/Sl stromal cell line derived from the Sl/Sl mouse has been genetically engineered to produce human IL-3 and human stem cell factor (SCF). The Sl/Sl (IL-3, SCF) cell line supports human myelopoiesis in LTC-IC assay. [3]

The M2-10B4 (IL-3, G-CSF) and Sl/Sl (IL-3, SCF) cell lines are provided by StemCell Technologies Ltd. on behalf of Dr. Donna Hogge, Terry Fox Laboratory (Vancouver, British Columbia) and cannot be sold or used for commercial purposes.

STORAGE:

Store cell lines at -135°C or colder, or in liquid nitrogen for up to 2 years.

MATERIALS:

Product	Unit Size	Catalogue Number
RPM1 1640	500 mL	36750
Dulbecco's Modified Eagle's Medium, High Glucose	500 mL	36250
Hanks Balanced Salt Solution Modified without Ca^{++} and Mg^{++}	500 mL	37250
Fetal Bovine Serum	100 mL 500 mL	06100 or 06200 06150 or 06250
Trypsin in Citrate Solution	100 mL	07400
Trypsin-EDTA Solution	100 mL	07901
MyeloCult® H5100	100 mL 500 mL	05100 05150
G418 (Geneticin)	200 mg	03812
Hygromycin B	100 mg	03813

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ADDITIONAL SUPPLIES REQUIRED:

Item	Suggested Suppliers
Dimethylsulfoxide (DMSO)	Sigma (D-2650)
Cryofreezing container	Nalgene (5100-001)

CULTURE MEDIA:

M2-10B4: RPMI 1640 with 10% Fetal Bovine Serum (FBS)
M2-10B4 (IL-3, G-CSF): RPM1 1640 with 10% FBS
Sl/Sl (IL-3, SCF): DMEM with 10% FBS

To ensure a continuous supply of the cell lines, a large number of vials should be cryopreserved from the original vial as soon as possible. Cells can be maintained in continuous culture for 3-6 months. The M2-10B4 (IL-3, G-CSF) and Sl/Sl (IL-3, SCF) cells should be checked periodically to ensure cells are producing expected levels of cytokines (by Elisa assay of supernatants).

CULTURE PROCEDURES:

Procedures should be performed in a biological safety cabinet certified for Level II handling. Universal handling precautions for biological samples should be used.

THAWING AND CULTURE OF CELLS:

1. Store cells at -135°C (or -80°C) until ready to use.
2. Place 10 mL of the appropriate culture media into a sterile 15 mL tube.
3. Thaw cells quickly by agitating vial in a 37°C waterbath.
4. Draw up the cell suspension in a 2 mL pipette and slowly add dropwise into the 15 mL tube containing culture medium.
5. Centrifuge at 1200 rpm (~250g) for 10 minutes.
   Wash step is required to remove DMSO.
6. Discard supernatant and resuspend the cells in 1-2 mL of culture media.
7. Perform a manual nucleated cell count and calculate the volume of cells required.
   Usually 2 x 10^5 cells per T75 cm^2 flask or 7 x 10^4 cells per T25 cm^2 flask is sufficient.
8. Make up the volume with the appropriate culture media.
   - T 25 cm^2 flask; 10 mL
   - T 75 cm^2 flask; 30 mL
9. Place the flask in a 37°C incubator with 5% CO_2 in air with 95% humidity.
10. Grow cells to confluence and passage cells every 7 – 10 days.

PASSAGE OF CELLS:

1. Scan the flasks using an inverted microscope to evaluate confluence. Split the flasks if they are close to or 100% confluent (every 7 - 10 days).
2. Remove the media from the flasks and discard.
3. Rinse flask with Hank's Balanced Salt Solution, Mg^{++} and Ca^{++} free to remove any FBS present. *FBS inhibits enzymatic reaction of trypsin.*
4. Add 1 mL of 0.25% Trypsin-citrate or 0.25% Trypsin-EDTA to each T25 cm^2 flask (2-3 mL per T75 cm^2 flask). Rotate flask to ensure complete coverage of the adherent layer with trypsin.
5. Place flask in 37°C incubator for 3-5 minutes.
6. Rock flask back and forth to determine if the adherent layer has lifted from flask surface. If so, add 0.2 mL of FBS to the flask to stop the trypsin reaction.
7. Pipette the cell suspension up and down a few times in the flask to disperse any cell clumps and prepare single cell suspension.
   If desired, cells can be washed twice using appropriate culture media.
8. Transfer 1/50 to 1/100 of the cell volume to a new T25 cm^2 flask or T75 cm^2 flask. Make up the volume as required.
   This seeding concentration should be sufficient for maintaining cells for approximately 7 to 10 days.
9. Place flasks in a 37°C incubator with 5% CO_2 in air and ≥95% humidity.

G418 AND HYGROMYCIN SELECTION:

Every second or third passage, the M2-10B4 (IL-3, G-CSF) and Sl/Sl (IL-3, SCF) flasks should be selected with G418 sulfate (also known as geneticin) and hygromycin B[2,3].

1. Prepare separate working solutions of 100 mg/mL G418 sulfate and Hygromycin B.
   Extreme care must be taken when handling hygromycin B as it is toxic (double-glove). Consult MSDS and follow precautions.

2. The following concentrations of G418 and Hygromycin B are used per flask:

M2-10B4 (IL-3, G-CSF)	G418, 0.4 mg/mL Hygromycin B, 0.06 mg/mL
Sl/Sl (IL-3, SCF)	G418, 0.8 mg/mL Hygromycin B, 0.125 mg/mL

   Example: How much G418 and Hygromycin is required for M2-10B4 (IL-3, G-CSF) cultured in T75 flask with 30 ml of media?

   0.12 mL of G418 at 100 mg/mL is required
   (30 mL x 0.4 mg/mL, need 12 mg of G418)

   0.018 mL of Hygromycin B at 100 mg/mL
   (30 mL x 0.06 mg/mL need 1.8 mg of Hygromycin B)

3. Add the appropriate volume of G418 and Hygromycin B to the fresh flask.
   G418 and hygromycin B should be mixed with the culture media before addition of cells.

FREEZING CELLS:

1. Cells are cryopreserved at a concentration of 1-2 x 10^6 cells per vial.
2. Label the required number of sterile 2 mL cryovials (1.8 ml capacity).
3. Prepare a 20% Dimethyl Sulfoxide (DMSO) solution in FBS.
   - Place FBS in culture tube and cool on ice.
   - Slowly add appropriate volume of DMSO and mix well.
   - Filter sterilize the solution using a 0.2 μm filter. Keep on ice.
4. Harvest cells and resuspend in cold FBS at twice the desired final cell concentration. *Example: Suspend at 4x10^6 cells/mL for cells cryopreserved at 2x10^6/mL per cryovial.*
5. Slowly add the 20% DMSO in FBS solution at a ratio of 1:1 to the tube containing cells. Continue to mix during the addition. Transfer 1 mL of cells in freezing medium to each cryovial. *The final cell suspension will be in 90% FBS:10% DMSO.*
6. Place cryovials immediately into freezing container. *To ensure good viability and cell recovery, do not let cells sit in freezing media at room temperature. Keep on ice and transfer within 15 minutes to the freezing container. Handle freezing container according to manufacturer's instructions.*
7. Place container in -70°C or -135°C freezer overnight.
8. Next day, remove frozen vials from the freezing container and store in –70°C freezer, -135°C freezer or in liquid nitrogen.

PREPARATION OF IRRADIATED M2-10B4 FEEDER LAYERS OR ENGINEERED M2-10B4:SL/SL

1. Trypsinize the desired feeder cells and wash the cells recovered twice in RPMI 1640/10%FBS or DMEM/l0%FBS.
2. Resuspend cells at 10^6-10^8 cells/mL in hLTCM (MyeloCult® H5100, Catalogue# 05100/05150) and irradiate cells with 8000 cGy from an X-ray or γirradiation source.
3. Dilute M2-10B4 cells to 1.5 X10^5 cells per mL in hLTCM or combine engineered M2-10B4 and Sl/Sl at 1: 1 ratio and dilute to 1.5 X10^5 (7.5x10^4 M2-10B4 and 7.5x 10^4 Sl/Sl) per mL in hLTCM. Place 2 mL (3x10^5 cells) per collagen-coated 35mm tissue culture dishes for LTC-IC assays in bulk culture or 0.1 mL (1.5x 10^4 cells) per well in 96-well flat-bottom culture plates for LTC-IC LDA assays.
4. Incubate at 37°C in 5% CO_2 in air in a humidified incubator. Incubate cultures for a minimum of 24 hours prior to the addition of test cells. Irradiated feeders can be used for up to 10 days later but if delays of >7 days are anticipated, the medium should be changed after the first 7 days.

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