LIPID MAPS THAWING AND PASSAGE PROCEDURE FOR RAW 264.7
Version 4, 4/22/05

The RAW 264.7 is a macrophage-like cell line derived from tumors induced in male BALB/c mice by the Abelson murine leukemia virus.

The RAW 264.7 cells to be used for the LIPID MAPS consortium were obtained from ATCC laboratories (cat# TIB-71; lot# 3002360), expanded to pass 3, and frozen down in aliquots. Freshly thawed cells are considered passage three, and the first passage of these cells is considered passage 4. Each aliquot (vial) of cells shall be passed for no more than 20 additional passages (total passage number 24) (1 vial per 2 months, 6 vials per year per lab).

To maximize uniformity across participating laboratories, RAW 264.7 cells should be thawed from original LIPID MAPS frozen stocks on the first Monday of odd months (Jan, March, May etc.). Passing cells twice per week as described below will result in maximum passage number of 24 passages. This passage number limit is based on significant changes in transfection efficiency beyond 25 passages. Transcriptional activation of COX2 in response to KDO lipid A is not measurably different in passage 7 and passage 25 cells. Maximal passage numbers could be subject to change if additional studies document variation in lipid components as a function of passage number.

The RAW 264.7 cells adhere to tissue culture-grade plastic through cation–dependent integrin receptors and other cation-independent receptors. They are extremely sensitive to lipopolysaccharide (LPS) endotoxin from gram-negative bacteria, therefore, only sterile disposable tissue culture ware and solutions, buffers, and media with endotoxin tested distilled deionized water should be used.

Thawing Procedure

1. Prepare growth medium (DMEM high glucose, 10% LIPID MAPS serum, 1% Pen/Strep) as defined by the RAW 264.7 Tissue Culture Reagents Table (see below). Do not filter medium. Assign a bar code to each bottle and enter into the LIMS.
2. Warm the growth medium for 15 to 20 minutes in a 37° C water bath.
3. Place 15 ml of the warmed fresh medium into a new 75 cm^2 flask.
4. Enter into LIMS, the barcode of the vial containing the frozen cells.
5. Thoroughly immerse the vial in 70% ethanol before thawing.
6. Thaw the cells by gently rubbing the vial between your hands (2-3min).
7. Carefully remove the cap and extract the cells with a pipette.
8. Dispense the cells in the newly prepared 75 cm^2 flask. Assign a barcode to the flask and enter into the LIMS.
9. Maintain the cells at 37° C, in a humidified atmosphere, with 5% CO_2. Calibrate the CO_2 monitor using the Fyrite kit every month.
10. After the cells have adhered, approximately 6 hrs, aspirate the old medium and add 15 ml of fresh medium.

11. When the cells are 80% confluent, follow the passage procedure below.

Passage Procedure

1. Warm the growth medium the same as in the thawing procedure.
2. Place 20 ml of the warmed fresh medium into new 150 cm^2 vessels.
3. Immediately after removing the parent T75 flask from 37˚ C and before aspirating the old medium from the cells, take an aliquot (1 ml) of medium for the TNFα assay. Assign a barcode, enter into LIMS and freeze the aliquot at -20˚ C.
4. Aspirate the old medium from the cells.
5. Rinse the cells 1 X with 5 ml DPBS.
6. Add fresh medium (e.g., 5 ml to 75 cm^2 flask, 10 ml to 150 cm^2 flask) to the cells.
7. Gently scrape the cells until all are dislodged.
8. Pipette the cell suspension, with a 10 ml pipette, up and down 5 X, without introducing bubbles.
9. Count the cells using a hemacytometer. A recommended counting procedure is as follows:
    a. Load 10 ul of the freshly suspended cells to the hemacytometer. Usually a 1:10 dilution in medium or DPBS of the suspended cells is needed before counting.
    b. The number of cells counted per square millimeter (middle 25 squares ruled on the hemacytometer or adjacent 16 squares, just be consistent) X dilution (if used) X 1 X 10^4
    = cell count per ml. This number X the total volume of the cell suspension = total cell count.
10. Dispense the cells into the new vessels. (e.g., 1 x 10^7 cells per 150 cm^2 flask). Assign a barcode and enter into the LIMS
11. Grow the cells to no more than 80% confluence, **2-3 days**, before passing.
12. At passage 12, immediately after removing the flask from 37˚ C and before aspirating the old medium, take an aliquot (1 ml) for the TNFα assay. Assign a barcode, enter into LIMS and freeze the aliquot at -20˚ C.

Experimental Procedure

Follow the passage procedure above, but use the recommended cell seeding and medium volume below to obtain approximately 80% confluence at **30 hours**.

1. **150 mm plates: 2 x 10^7/20 ml medium**
2. **100 mm plates: 5 x 10^6/10 ml medium**
3. **60 mm plates: 2 x 10^6/5 ml medium**
4. **6-well plates: 8 x 10^5/well/3 ml medium**
5. **12-well plates: 4 x 10^5/well/2 ml medium**
6. **24-well plates: 2 x 10^5/well/1 ml medium**
7. **96-well plates: 5 x 10^4/well/100 ul medium**
8. **150 cm^2 flask: 2 x 10^7/20 ml-30 ml medium**
9. **75 cm^2 flask: 1 x 10^7/10 ml-15 ml medium**

10. 25 cm^2 flask: 3 x 10^6/5 ml-7 ml medium

RAW 264.7 Tissue Culture Reagents – Growth Media

Reagent	Source	Catalog No.	F.W. or Stock Conc.	Quantity	Final Conc.
Dulbecco's Modified Eagle's Medium (DMEM)	Cellgro	10-013	1X DMEM with 4.5g/l Glucose and 4mM L-Glutamine	445 ml	
Heat-inactivated fetal calf serum (FCS)	Hyclone	SH30071.03 ANG19242	100%	50 ml	10%
Antibiotic Penicillin Streptomycin	Gibco	15140-122	100%	5 ml	1%
Dulbecco's Phosphate-Buffered Saline (DPBS)	Cellgro	21-031	100%		

To heat-inactivate the serum:
1. Thaw at 4° C.
2. Heat at 56° C for 30 minutes.
3. Aliquot serum in 50 ml tubes and store at 4° C until use.

CELLGRO 800-235-5476
GIBCO/INVITROGEN 800-955-6288
HYCLONE 800-492-5663