Core E - LIPID MAPS THAWING AND PASSAGE PROCEDURE FOR RAW 264.7
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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 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_2-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 sterilized disposable tissue culture ware and solutions, buffers, and media prepared 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) according to protocol PS0000000901.
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 the LIMS *Cell Line – Thaw* application the barcodes of the protocol, vial of frozen cells (freeze), and medium. The application returns the barcode ID for the new vessel.
5. Thaw the cells by immersing the bottom of the tube in a 37 °C water bath and rotating the tube for 1-2 min.
6. Thoroughly wipe the outside surfaces of the vial with 70% ethanol.
7. In a laminar flow hood, carefully remove the cap and extract the cells with a pipette.
8. Dispense the cells in the newly prepared 75 cm^2 flask. Label with the barcode assigned in Step 4.
9. Maintain the cells at 37 °C, in a humidified atmosphere, with 5% CO_2. (Calibrate the CO_2 monitor monthly using a Fyrite kit.)
10. After the cells have adhered, 2 hr to overnight, 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 flask from the 37 °C incubator and before aspirating the old medium from the cells, take an aliquot (1 mL) of medium for the TNFα assay. Label the sample for TNFα with the barcode ID for the vessel. Print from the *Thawing Procedure* and edit the first or second line to include the notation "TNF". Freeze the aliquot at -20 °C.
4. Aspirate the old medium from the cells.
5. Add fresh medium (10 mL to 150 cm^2 flask) to the cells.
6. Gently scrape the cells with a sterile scraper until all cells are dislodged from all flask surfaces.
7. In order to dislodge clumps of cells, aspirate the whole cell suspension and deliver the stream rapidly against the side of the flask at approximately a 90° angle. Repeat at least 10 times, while minimizing the introduction of bubbles.
8. Count the cells using standard techniques. The Core E procedure is to count cells in an Improved Neubauer hemacytometer.
9. Dispense the cells into the new vessels (2 x 10^6 cells per 150 cm^2 flask).
Two separate times, enter into the LIMS *Cell Line – Passage* application the barcodes of the protocol, parent flask and medium, vessel size and other data. The first time, for the vessel used for cell passage, the application returns a barcode ID for the new passage (daughter) vessel. The second time, for plating cells in Kdo_2-lipid A experiments, enter the plate size and "Total vessels = 1" to obtain the barcode for use in the appropriate protocol for treatment with Kdo_2-lipid A. This procedure generates barcode ID's with suffixes A (cell maintenance) and B (experimental procedure) for the same passage.
10. Grow the cells to no more than 80% confluence (this requires up to 30 hr) before passing.
11. At passage 12, immediately after removing the flask from 37° C and before aspiration of the old medium, take an aliquot (1 mL) for TNFα assay. Label the sample for TNFα assay with the barcode ID for the vessel. Print the label from this procedure and edit the first or second line to include the notation "TNF". 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.

Core E uses 100 mm plates for Kdo_2-lipid A treatments and 150 cm^2 flasks for cell passage:

100 mm plates: 5 x 10^6 / 10 mL medium
150 cm^2 flask: 2 x 10^6 / 30 mL medium