PROPAGATION & CULTURING OF RAW264.7 CELLS

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BACKGROUND

These cells are macrophage-like cell line derived from Balb/c mice. They maintain many of the properties of macrophages including NO production, phagocytosis (beads, other), extreme sensitivity to TLR agonists and motility. They are susceptible to genetic drift so freezer stocks must be made from early passage number cells. They are genetically fairly heterogeneous, which can be a benefit as clones that have reduced binding to certain ligands can be isolated and propagated. They express SRA but not MARCO. They may be transfected, although mortality is high and expression is not guaranteed.

NOTES

Freezer Stocks: These cells are fairly resistant to freeze/thaw.

Propagation: These cells should never be completely confluent (60-75% max). In general macrophages do not like to be too dense. These cells are known to suffer from genetic drift – they are genetically heterogeneous and there will be subclones within the mix that differ in receptor expression, TLR responsiveness, etc. To avoid genetic drift use low passage numbers (i.e. less than 20) and ensure that all cells (or almost all) are lifted during subculture. Lifting these cells can be tricky (they are macrophages after all), trypsin EDTA is a possibility, lidocaine + EDTA works sometimes and scraping is possible.

Culturing stably expressing cell lines: Stable expression is hard to achieve in this cell line and cells must be continuously tested for expression. When taking stably expressing cells (usually because transfected with linearized construct in pcDNA3.1), start culture at 0.2mg/ml G418, after 2 days increase to 0.6mg/ml).

EQUIPMENT

- Equipment:
  - Level 2 BioHood
  - 37°C, 5% CO2 Incubator
  - Centrifuge
- Materials:
  - ATTC DMEM + 10% FCS (+L-glutamine if necessary, Pen/Strep is optional). **Note:** RPMI + 10% FCS (+L-glutamine if necessary, Pen/Strep is optional) can also be used.
  - PBS (heated to 37°C)
  - Trypsin-EDTA

Protocol

ATCC says: For a 75 cm^2 flask, remove all but 10 ml culture medium (adjust amount accordingly for other culture

vessels). Dislodge cells from the flask substrate with a cell scraper; aspirate and add appropriate aliquots of the cell suspension into new culture vessels.

Note: scraping ensures that all the cells come off, but may lead to lysis and loss of cells. **I say:**

1. When subculturing cells (60-75% confluency), discard old media from flask.
2. Wash once with PBS.
3. Add appropriate volume of trypsin-EDTA (i.e. 10 ml in $T175^2$ flask) and incubate at 37**°**C for 5-15 min, and ensure that cells are lifting. *You may bang the flask to help them along*.
4. a) If they are lifting, then remove the cell suspension into a centrifuge tube with about 2x the volume of complete media (i.e. serum in order to stop the reaction) and continue with centrifugation.

   b) If they are not lifting, proceed to scraping. Spin for 5' x 1000 rpm. Resuspend in fresh media & seed subsequent flasks with a dilution of approximately 1:3 to 1:6.

To Freeze cells: Grow to a density of 1 x 10E6 cells in 20 mL of media. Centrifuge at 1000rpm x 5 min. Resuspend in 3.6mL of FCS and add 400uL of DMSO in a drop-wise manner. Pipette 1mL into a cryovial. Put into a freezing bomb & store at -80˚C overnight. Transfer to liquid nitrogen or keep in -80˚C for up to 6 months.

To Defrost cells: Warm media (see recipe above) to 37˚C. Pipette 30 mls into a 50 ml conical tube and keep warm. Take a vial of frozen cells and defrost as quickly as possible (e.g. but swirling in the 37˚C water bath) until just defrosted. Pour the contents into the warmed media and centrifuge at 1000 rpm x 5 min. This removes the DMSO. Resuspend in 15 mL media & incubate overnight. Check cell density the next day & adjust accordingly.