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## Tissue culture protocol for RAW264.7

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

Lewis lab protocol for culturing RAW264.7 cells.

## Materials

- DMEM (31966047, Gibco)
- 10 % heat-inactivated FCS

## Troubleshooting



## Subculturing

- 1 Remove the medium and add 5 ml of fresh medium to 75 cm<sup>2</sup> flask.
- 2 Scrap cells with a cell scraper.
- 3 Aliquot cell suspension into new flask at a ratio of 1:5 to 1:10.
- 4 Incubate the culture at 37°C, 5% CO<sub>2</sub> in a suitable incubator.
- 5 Subculture every 2 to 3 days.

## Thawing

- 6 Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
- 7 Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.
- 8 Centrifuge the cell suspension at approximately 300 x g for 5 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium and transfer the cells to an appropriate size vessel.
- 9 Incubate the culture at 37°C, 5% CO<sub>2</sub> in a suitable incubator.

## Freezing

- 10 Freeze cell suspension in complete growth medium supplemented with 10 % (v/v) DMSO at 1 to 5 × 10<sup>6</sup> cells/ml.