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Tissue culture protocol for RAW264.7

In 1 collection

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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^2 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% CO2 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% CO2 in a suitable incubator.

Freezing

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