Unthawing RAW264.7 cells

Tyler C. Moore

Abstract

RAW264.7 cells are a macrophage-like cell line derived from BALB/c mice. These cells are loosely adherant and can be detached from cell culture flasks by light shaking or use of TrypLE (or Trypsin/EDTA).

Citation: Tyler C. Moore Unthawing RAW264.7 cells. **protocols.io**
dx.doi.org/10.17504/protocols.io.nxpdfmn
Published: 20 Mar 2018

Guidelines

Only thaw cultures from frozen vials when there are no active cultures in the incubator. Keep cells sterile by wearing gloves, ethanol treating your hands and surfaces, using sterile pipette tips, and ***only opening vials with cells in the BSL-2 laminar flow hood***.

Materials

- Freeze Media (FBS 10% DMSO) by Contributed by users
- RAW264.7-LuciaISG-WT by Contributed by users
- RAW264.7-LuciaISG-IRF3KO by Contributed by users
- T25 or T75 Flask by Contributed by users
- Complete DMEM (DMEM, 10% HI FBS, 50ug/mL gentamycin) by Contributed by users

Protocol

Step 1.

Pre-warm Complete Media in 37 degree C water bath or in the CO2 incubator (space permitting)

REAGENTS
- Complete DMEM (DMEM, 10% HI FBS, 50ug/mL gentamycin) by Contributed by users

Step 2.

Thaw the vial. Gentle agitation in a 37 deg C water bath. You can also hold the vial tightly in your

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

hand. Check the label to ensure you have the correct cell line. Avoid getting the vial wet to avoid contamination.

Step 3.
Decontaminate vial by spraying with 70% ethanol.

Step 4.

Transfer Cells.
In the hood: Use a micropipette (with sterile tip) to transfer cell suspension to sterile 15mL tube containing 15mL pre-warmed complete DMEM.

Step 5.

Centrifuge Cells.

Spin cells at 200-300x g (a.k.a. RCF) for 5 minutes. Make sure the centrifuge is balanced.

Step 6.

Remove supernatant.

Decant into a beaker in the laminar flow hood. Resuspend cells in 1mL Complete DMEM.

Step 7.

Transfer the vial contents to a flask.

Use a serological pipette to transfer the resuspended cells to a flask containing 5mL Complete Media (T25) or 15mL Complete Media (T75)

Step 8.

Inspect cell density and viability under the inverted microscope.

Step 9.

Place culture in 37 deg C 5% CO2 incubator.

Step 10.

Slightly loosen cap of flask to allow gas exchange.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited