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Culturing RBL-2H3 rat basophils

In 1 collection

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We use this protocol and it's working

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Abstract

Guidelines for thawing, maintaining, subculturing, and freezing RBL-2H3 (rat basophil) line.

Materials

RBL-2H3 rat basophil cells **ATCC Catalog #**CRL-2256
T75 flasks for cell culture **Thermofisher**
Mast Cell Degranulation Assay Kit **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**IMM001
Eagles Minimum Essential Medium **VWR International (Avantor) Catalog #**MSPP-302003
Heat-inactivated fetal bovine serum **Thermofisher Catalog #**A3840101
70% ethanol **VWR International (Avantor)**
Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D2438
T25 flasks
IMDM **Gibco - Thermo Fisher Scientific Catalog #**12440053
PenStrep **Invitrogen - Thermo Fisher**
1-Thioglycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6145
Trypsin-EDTA (0.25%) **Thermo Fisher Scientific Catalog #**25200056
PBS
15 mL centrifuge tubes

Protocol materials

- 70% ethanol **VWR International (Avantor)**
- PenStrep **Invitrogen - Thermo Fisher**
- 1-Thioglycerol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M6145
- RBL-2H3 rat basophil cells **ATCC** **Catalog #**CRL-2256
- T75 flasks for cell culture **Thermofisher**
- Trypsin-EDTA (0.25%) **Thermo Fisher Scientific** **Catalog #**25200056
- Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D2438
- Mast Cell Degranulation Assay Kit **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**IMM001
- Eagles Minimum Essential Medium **VWR International (Avantor)** **Catalog #**MSPP-302003
- Heat-inactivated fetal bovine serum **Thermofisher** **Catalog #**A3840101
- IMDM **Gibco - Thermo Fisher Scientific** **Catalog #**12440053

Thawing the RBL-2H3 rat basophil cell line

8m

1 15m

To make the complete culture medium for these cells, add Heat-inactivated fetal bovine serum **Thermofisher Catalog #**A3840101 to a final concentration of [M] 15 % (v/v) in Eagles Minimum Essential Medium **VWR International (Avantor) Catalog #**MSPP-302003. Add PenStrep **Invitrogen - Thermo Fisher** to a final concentration of 1×. Sterile-filter the media under strict aseptic conditions.

It's important to avoid excessive alkalinity of the medium during recovery of the cells. Prior to adding cells, you should place the culture vessel containing the complete growth medium into the incubator for at least 00:15:00 to allow the medium to reach pH 7.0 – pH 7.6.

2 7m

The frozen cell culture should have been stored in liquid nitrogen and not at -70 °C, as storage at -70 °C will result in loss of viability. To use the cells, first thaw the vial by gentle agitation in a 37 °C water or bead bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water (if using). Thawing should be rapid (approximately 00:02:00 for water bath, 00:05:00 for bead bath).

3

Remove the vial from the water bath as soon as the contents are thawed, and decontaminate the outside of the vial by dipping in or spraying with 70% ethanol **VWR International (Avantor)**. All of the operations from this point on should be carried out under strict aseptic conditions.

4 5m

Working quickly to avoid loss of viability, transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g, 00:05:00.

5

Resuspend cell pellet with the recommended complete medium (see the specific batch information for the recommended dilution ratio) and dispense into a 75 cm^2 culture flask.

6 Incubate the culture at 37 °C in a suitable incubator with a 5% CO_2 in air atmosphere.

Subcultivation

10m

7 Subculture the cells every 2–3 days. We recommend a subcultivation ratio of 1:4 to 1:8.

7.1 Remove and discard used culture medium.

7.2 Rinse the cell layer with Trypsin-EDTA (0.25%) **Thermo Fisher Scientific Catalog #**25200056 or PBS to remove traces of serum.

7.3 Add 2.0 mL to 3.0 mL of Trypsin-EDTA (0.25%) **Thermo Fisher Scientific Catalog #**25200056 solution to the flask. Incubate cells at 37 °C until they detach — for RBL-2H3, this is usually within 00:10:00 .

10m

7.4 Add 6.0 mL to 8.0 mL of complete growth medium (≥ 2× the trypsin volume) and dislodge remaining cells in the flask by gently pipetting.

7.5 Add appropriate aliquots of the cell suspension to new culture vessels and incubate the culture at 37 °C in a suitable incubator. We recommend 5% CO_2 in air atmosphere.

Cryopreservation

8m

8 Cryopreserve many aliquots of low-passage cells for future experiments.

8.1 Recover cells from flask as in 7.1–7.4.

8.2 Count cells. Prepare a mixture of 10 µL cells and 10 µL trypan blue, inverting cells gently before removing sample for counting. Mix well, then transfer 10 µL trypan-diluted cells to a Countess slide. Count using Countess to determine cell density and relative viability.

8.3 Freeze cells at ~1 million live cells in 1 mL cryo-media per vial. Ensure there are enough live cells for the desired number of aliquots. Centrifuge cells to be frozen at 125 x g, 00:05:00 . 5m

8.4 Meanwhile, prepare cryo-media by diluting

Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D2438

in complete growth medium to a final concentration of [M] 10 % (v/v) .

8.5 After centrifugation, aspirate supernatant. Resuspend cell pellet in cryo-media. Working quickly to avoid loss of viability, aliquot cells to pre-labeled screw-top cryotubes.

8.6 Place cells in a Mr. Frosty or similar container that controls freezing rate. Place container at -80 °C Overnight . 10m

8.7 Transfer frozen cells to vapor-phase liquid nitrogen for long-term storage.

Protocol references

HMC1.2 Manufacturer Guidelines:
https://www.emdmillipore.com/US/en/product/HMC-1.2-Human-Mast-Cell-Line,MM_NF-SCC062?ReferrerURL=https%3A%2F%2Fwww.google.com%2F#documentation

RBL-2H3 Manufacturer Guidelines:
https://www.atcc.org/api/pdf/product-sheet?id=CRL-2256