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Culturing HMC1.2 human mast cells

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We use this protocol and it's working

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Abstract

Guidelines for thawing, maintaining, subculturing, and freezing the HMC1.2 (human mast cell) 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

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

⊗ 70% ethanol **VWR International (Avantor)**

⊗ Heat-inactivated fetal bovine serum **Thermofisher Catalog #A3840101**

⊗ 1-Thioglycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M6145**

⊗ PenStrep **Invitrogen - Thermo Fisher**

⊗ IMDM **Gibco - Thermo Fisher Scientific Catalog #12440053**



Thawing the HMC1.2 human mast cell line

8m

- 1 To make the complete culture medium for these cells, add
 Heat-inactivated fetal bovine serum **Thermofisher Catalog #A3840101** to a final concentration of **1M** 10 % (v/v) ,
 1-Thioglycerol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M6145** to a final concentration of **1M** 1.2 millimolar (mM) , and
 PenStrep **Invitrogen - Thermo Fisher** to a final concentration of 1× in
 IMDM **Gibco - Thermo Fisher Scientific Catalog #12440053** . Sterile-filter the media under strict aseptic conditions.
- 2 You should have kept the frozen cell culture 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 it in or spraying it with
 70% ethanol **VWR International (Avantor)** . Carry out all operations from this point on under strict aseptic conditions.
- 4 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
 300 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 25 cm² culture flask.
- 6 Incubate the culture at 37 °C in a suitable incubator with a 5% CO₂ in air atmosphere.

7m

5m

Subcultivation



- 7 Subculture the cells every 2–3 days when cell density reaches 1.0–1.5 million live cells/mL. The recommended seeding density for subculturing is 250,000 live cells/mL.
- 7.1 Transfer cells to a 15 mL tube. Dislodge any cells from the flask by gently pipetting.
- 7.2 Add appropriate aliquots of the cell suspension to new culture vessels and incubate the culture at 37 °C in a suitable incubator with a 5% CO₂ 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.
- 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 Cells will be frozen 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 300 x g, 00:05:00 .
- 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 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 .

5m

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>