RESGRO™ Culture Medium
500 mL

Catalog No. SCM002

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

RESGRO Culture Medium is a complete ready-to-use product that can be used to complement traditional murine Embryonic Stem (ES) cell culture media containing ESGRO® mouse LIF medium supplement. In contrast to routine ES cell culture using ESGRO supplemented medium, RESGRO Culture Medium is recommended for a number of specialized applications.

Rescue of established ES cell lines
RESGRO Culture Medium has the capacity to rescue established ES cell lines that have started drifting, and either generate low percentage chimeras or have lost germline transmission capability. Differentiation, which is present in the ES cells but not visible with traditional medium, will become recognizable when using RESGRO Culture Medium. After 2 passages, a clear difference is seen between differentiated and undifferentiated ES cells, at which time undifferentiated cells can be selected for by sub-cloning.

Murine ES cell derivation
The efficiency of ES cell derivation is greatly strain dependent. To date, very few murine ES cell lines are available from inbred strains other than 129 strains, and those derived have generally been obtained with low success rates. Furthermore, ES cells derived from other strains than 129 are in general more difficult to propagate *in vitro*. Especially at high passage number and after genetic manipulation, these cell lines generate chimeras less efficiently and contribute less frequently to the germline.

RESGRO Culture Medium enables the efficient derivation and maintenance of ES cell lines from several inbred mouse strains, including certain strains that were previously considered to be non-permissive for ES cell derivation. A recent study demonstrated that RESGRO medium allowed the derivation of ES cell lines from inbred strains other than 129 (including FVB, a strain previously considered to be non-permissive for ES cell derivation and C57Bl/6N, BALB/c, 129/SvEv and DBA/2N mouse strains)[1].

Culture of ES cells without a fibroblast feeder layer
RESGRO Culture Medium allows for the culture of ES cells on bare (gelatinized) culture dishes. Even in the absence of a fibroblast feeder layer ES cells maintain their undifferentiated character and their germline transmission capability for at least 5 passages, when cultured with RESGRO Culture Medium. After trypsinization pure ES cell suspensions without fibroblast cells can be obtained. Fibroblast cells will no longer interfere during blastocyst injections, diploid aggregations, tetraploid aggregations and electroporations.

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Kit Components

1. RESGRO Culture Medium: (Catalog No. SCM001) Two (2) 250 mL bottles.

Materials Not Supplied

1. Sterile L-Glutamine solution (200 mM) (Catalog No. TMS-002-C)
2. Stericup-GP Filter Unit, 0.22 µm, polyethersulfone, 250 mL (Catalog No. SCGPU02RE)
3. Trypsin-EDTA (Catalog No. SM-2003-C)

Storage

Upon receipt, RESGRO Culture Medium should be stored at -80℃ until required for use. When stored properly, product is stable until the expiration date stated on the label. Once opened, the unused portion can be stored at 4℃ for at least 4 weeks. Protect from light during storage. Avoid repeated freeze/thaw cycles.

It is recommended that the product be discarded following expiry. The addition of certain supplements can affect product stability and characteristics. Turbidity and flocculent material may be present after thawing or after prolonged freezer and/or refrigerated storage.

Preparation Before Use

There is no need to test RESGRO medium before use. Prior to use, RESGRO Culture Medium needs the addition of L-Glutamine and the medium should be examined for changes in pH indicated by a change in color. The appearance of slight precipitates or particulates in the solution will not deleteriously affect the performance of the product.

Prepare the RESGRO Culture Medium solution by adding 5 mL of sterile L-Glutamine (200 mM) to each 250 mL bottle of RESGRO Culture Medium. If turbidity or flocculent material is observed, we recommend filtering the media using standard cellulose acetate, PVDF or PES tissue culture filters.

Production and Quality Control

All components and the complete medium are tested in advance. The solution is 0.22 micron filtered and embryo-tested. Endotoxin level is less than 10 EU / mL. Only compounds with the highest quality and purity standards are used in the production of RESGRO Culture Medium. The efficiency of ES cell derivation with C57BL/6N blastocysts is tested for each batch. A derivation efficiency of at least 25% is required for batch approval.

Rescue of ES Cell Lines

RESGRO Culture Medium has the capacity to rescue traditional ES cell lines that have started drifting and either generate low percentage chimeras or have lost germline transmission capability. Differentiated ES cells are not visible with traditional ES cell culture medium, but will become visible with RESGRO medium. After 2 passages, a clear difference is seen between differentiated and undifferentiated ES cells. At that moment, it is recommended to perform a subcloning to pick out the undifferentiated cells. **The selection procedure should be repeated if some differentiation is still present after one subcloning procedure.**

1. Culture the ES cells in RESGRO Culture Medium for 2 passages on a monolayer of mitotically inactivated mouse embryonic fibroblast cells.
2. After 2 passages, replate 1/3 - 1/5 of the cell suspension on the same dish without inactivated mouse embryonic fibroblast cells.
3. After 2 days, a clear difference will be observed between **3-dimensional (undifferentiated)** and **flat growing (differentiated)** colonies. By tapping the dish, the 3-dimensional colonies (undifferentiated) will detach.
4. **Collect the supernatant (which will contain the undifferentiated cells) and discard the dish containing the differentiated cells**.
5. Centrifuge the supernatant and remove the medium.
6. Add 0.5 mL of trypsin/EDTA to the cell pellet.
7. Pipette up and down with a 1 mL pipette (do not use pipette tip of smaller volume).
8. Place the cell suspension in a water bath at 37°C for 1.5 minutes.
9. Pipette up and down, 10 times (with a 200 µL pipette tip or a 1 mL pipette).
10. Add 9.5 mL of RESGRO Culture Medium.
11. Centrifuge and remove the supernatant
12. Add an appropriate volume of RESGRO Culture Medium, which will depend upon the final volume that you prefer to plate the cells. For 6-well plates, it is recommended that the cells be suspended in 4 mL of RESGRO Culture Medium. Plate 1/3 - 1/6 of the ES cells on wells containing mitotically inactivated mouse embryonic fibroblast cells. Alternatively, ES cells can be cultured in medium containing ESGRO mLIF supplement.

    ***Note**: Avoid contact between the colonies! If the ES cells have been plated at too high a density, replate ES cells at a lower density the following day.*

Derivation of ES Cell Lines

This protocol is based upon that used by Schoonjans L. *et al.* (2003). Please refer to this reference for comprehensive details on the application of RESGRO Culture Medium for ES cell derivation.

1. Collect 3.5 to 4.5 day old blastocyst stage mouse embryos and plate on a 96-well dish covered with a freshly prepared monolayer of mitotically inactivated mouse embryonic fibroblast feeder cells.
2. During the first 2 days remove only 3/4 of the medium and replace gently with fresh RESGRO Culture Medium (in order to avoid detachment of the blastocysts).
3. After attachment of the blastocysts, replace the medium completely on a daily basis with RESGRO Culture Medium.
4. After 5-6 days in culture, remove the inner cell mass (ICM) outgrowth from the trophoectoderm. Replate the cells following trypsinization with 0.25% trypsin/1 mM EDTA on a 96-well dish covered with a monolayer of mitotically inactivated mouse embryonic fibroblast feeder cells.
5. Culture the ES cells until subconfluency, and then replate on larger culture dishes.
6. Passage ES cells every 2-4 days on freshly prepared feeder layers, and replace with fresh RESGRO Culture Medium daily.

Establishing an ES Cell Culture from Frozen Stock

1. Dispense 3 mL of RESGRO Culture Medium into a 6-well plate covered with mitotically arrested mouse embryonic fibroblast cells and place in a 37°C incubator for 1 hour.
2. Thaw a frozen vial of ES cells in a water bath at 37℃. Remove the vial just before the last trace of ice has melted.
3. Gently pipette the content of the vial up and down several times (removal of the cryoprotective medium is not necessary).
4. Dispense the cells into the wells of the 6-well plate containing RESGRO Culture Medium.
5. Gently disperse the cells by shaking to ensure a homogenous distribution.
6. Disperse cells again after 35 minutes and 1 hour by gently shaking.
7. Following attachment of the ES cells to the feeders (2-3 hours), gently remove the RESGRO Culture Medium and replace it with 4 mL of fresh RESGRO Culture Medium.
8. Replace the RESGRO Culture Medium daily with fresh medium. Ensure that ES cell colonies do not come into contact with each other by following the passaging protocol listed below.

<u>Other well sizes and suggested volumes of medium:</u>

96-well: 200 µL
48-well: 500 µL
24-well: 1 mL
12-well: 1.5 mL
6-well: 4 mL
10 cm: 10 mL

ES Cell Passage

It is recommended that ES cells be passaged every 2-3 days.

1. Remove RESGRO Culture Medium.
2. Dispense 0.5 mL of trypsin-EDTA into each well of the 6-well plate.
3. Remove the trypsin immediately.
4. Dispense an additional 0.5 mL of trypsin into each well of the 6-well plate.
5. Incubate the plate for 2 minutes at 37℃.
6. Pipette up and down using a 200 µL pipette tip until a single cell suspension is obtained.
7. Add 5 mL of RESGRO Culture Medium to the well and transfer the cell suspension to a 15 mL tube.
8. Rinse the 6-well plate with an additional 4.5 mL of RESGRO Culture Medium and add to the 15 mL tube. Total volume will be 10 mL.
9. Centrifuge for 5 minutes at 1100 rpm and aspirate the supernatant.
10. Split the ES cells 1/4 to 1/8 ($\pm$0.5 10^6 cells per 6-well) using RESGRO Culture Medium.

ES Cell Cryopreservation

1. Aspirate the RESGRO Culture Medium.
2. Dispense 0.5 mL of trypsin-EDTA into each well of the 6-well.
3. Aspirate the trypsin immediately.
4. Dispense an additional 0.5 mL of trypsin into each well of the 6-well plate.
5. Incubate the plate for 2 minutes at 37℃.
6. Pipette up and down using a yellow tip until a single cell suspension is obtained.
7. Add 5 mL of RESGRO Culture Medium to one well and transfer the cell suspension to a 15 mL tube.

8 Rinse the 6-well plate with an additional 4.5 mL of RESGRO Culture Medium and add to the 15 mL tube. Total volume will be 10 mL.

9. Centrifuge for 5 minutes at 1100 rpm and aspirate the supernatant.
10. Resuspend the cell pellet in freezing medium and aliquot in cryovials.
11. Transfer cryovials to -80℃ to freeze in contai ners filled with isopropanol.
12. Transfer to liquid N_2 tank.

<u>Freezing medium</u>
4 mL RESGRO Culture Medium
6 mL Fetal Bovine Serum
20 mL Cryoprotective medium (Basal Eagle's medium with Hanks' BSS and 15% dimethylsulfoxide without L-Glutamine).

The freezing medium can be stored at 4℃ for 3 days

References

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