MimEX™ GI Expansion Media

Media for the Expansion of Adult Gastrointestinal Stem Cells

Catalog Number: MIM003

Volume: 250 mL

PRODUCT DESCRIPTION

The MimEX™ GI Tissue Model System allows for the expansion and differentiation of ground-state, adult stem cell populations from the gastrointestinal tract. These cells create sustainable and accessible 3-dimensional gastrointestinal tissues *in vitro* and provide a valuable tool for biomarker discovery, disease modeling, drug screening, and developmental biology studies. The MimEX™ GI Expansion Media component of this system will expand adult stem cell populations of the gastrointestinal tract under clonal conditions while maintaining regional identity, genome stability, and disease state (1,2).

INTENDED USE

MimEX™ GI Expansion Media is formulated to support the expansion of adult epithelial stem cells derived from the gastrointestinal tract. The media has been tested for its ability to support expansion of adult human intestinal stem cells *in vitro* when grown on plates coated with MimEX™ Irradiated Fibroblasts.

STABILITY & STORAGE

Upon receipt, this media should be stored at ≤ -20 °C in a manual defrost freezer. The media can be thawed at 2-8 °C or at room temperature. Thawed media can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer for up to 3 months or used within 2 weeks when stored at 2-8 °C. Avoid repeated freeze-thaw cycles.

PRECAUTIONS

When handling biohazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- These reagents should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations among adult stem cell lines originating from different donors.

REFERENCES

1. Wang, X. *et al.* (2015) Nature **522**:173.
2. Yamamoto, Y. *et al.* (2016) Nature Comm. **7**:10380.

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PROCEDURE FOR THE EXPANSION OF HUMAN ADULT GASTROTINTESTINAL STEM CELLS

The protocol below describes the expansion of human adult gastrointestinal (GI) stem cells in MimEX™ GI Expansion Media.
Note: *This protocol must be read in its entirety before using this product.*

OTHER MATERIALS REQUIRED

- GI Adult Stem Cells (purchased directly from R&D Systems® or isolated from tissue using the MimEX™ Tissue Processing Kit (R&D Systems®, Catalog # MIM001)).
- MimEX™ Irradiated Fibroblast Kit (R&D Systems®; Catalog # MIM005).
- DMEM/F12.
- Penicillin-Streptomycin (100X), optional.
- 0.05% Trypsin/EDTA.
- Sterile Phosphate-Buffered Saline (PBS) (Tocris®; Catalog # 3156).
- 6-well tissue culture treated plates.
- 15 and 50 mL centrifuge tubes.
- Serological pipettes.
- Pipette and pipette tips.
- 37 °C and 7.5% CO_2 humidified incubator.
- Centrifuge (low speed clinical or equivalent).
- Hemocytometer.
- Inverted Microscope.
- Water bath.

REAGENT PREPARATION

MimEX™ GI Expansion Media - Thaw the Expansion Media at 2-8 °C or room temperature. Add Penicillin-Streptomycin at a 1:100 dilution. Store under sterile conditions at 2-8 °C for up to 2 weeks.

Note: *If Penicillin-Streptomycin is not needed for the experiment, it can be omitted.*

PROCEDURE

Preparing Plates with MimEX™ Irradiated Fibroblasts

The following protocol is to plate one vial of MimEX™ Irradiated Fibroblasts into 2 wells of a 6-well plate. If an alternate plate size is desired, adjust volumes and cell numbers accordingly.

1. Place the 6-well plate at 2-8 °C for 10 minutes.
2. Dilute Cultrex® Stem Cell Qualified BME 1:5 in ice cold DMEM/F12. Keep on ice. Diluted Cultrex® BME can be stored at 2-8 °C for up to 1 week.
3. Coat wells with diluted Cultrex® BME mixture. Pipette 1.0 mL into the center the well, swirl gently to coat the entire well, and remove by pipette while tilting the plate. Ensure there are no air bubbles during the coating. Quickly add the volume to the second well and repeat for all wells needed. Return the diluted Cultrex® BME mixture to ice every 3-4 wells to keep it cold and prevent premature polymerization.
4. After all wells are coated, tilt the plate at an angle for 15 seconds. Using a pipette remove and discard any remaining Cultrex® BME that pools at the bottom of the wells.
5. Incubate the plate at 37 °C/7.5% CO_2 for 10 minutes. At the end of the incubation, the wells have a shine when held at an angle in light. Do not allow the plates to become dry.
6. While the Cultrex® BME-coated plates are incubating, retrieve the needed vials of MimEX™ Irradiated Fibroblasts from liquid nitrogen storage. Thaw in a 37 °C water bath.
7. Aliquot the needed volume of MimEX™ Fibroblast Media (2 mL/well) into a 50 mL centrifuge tube. Each vial of MimEX™ Irradiated Fibroblasts is enough to coat 2 wells of a 6-well plate, or the equivalent surface area.

PROCEDURE *CONTINUED*

8. Add 0.5 mL of MimEX™ Fibroblast Media to the cryovial of fibroblasts and gently pipette to re-suspend. Slowly add the cells dropwise to the media in the 50 mL centrifuge tube, with gentle swirling. Do not pipette up and down vigorously. **Note:** *Vigorous treatment of MimEX™ Irradiated Fibroblasts may impact viability and performance.*
9. Rinse the cryovial once with 0.5 mL of the resuspended fibroblasts. Slowly add the media back to the 50 mL centrifuge tube dropwise while mixing by gentle swirling.
10. Add the fibroblast suspension to the coated wells, 2.0 mL/well. Ensure there are no air bubbles in the wells.
11. **Critical Step:** Evenly distribute the cells in the well by the **MimEX™ Cell Plating Procedure.**
    a. Place the plate in the incubator. Allow media to become still.
    b. Slide the plate forward and backward evenly three times. You should observe a wave traveling forward and back. Avoid swirling the media.
    c. Allow the media to become completely still (about 3 seconds).
    d. Slide the plate side to side evenly three times. A wave should be observed traveling side to side. Avoid swirling the media.
    e. Allow the media to become completely still (about 3 seconds).
    f. Repeat steps a-e two more times (for a total of three times).
12. **Critical Step:** Incubate the plate in a **37 °C/7.5% CO_2** incubator overnight **without disturbing the plate.**
13. As early as possible the next day check the density and distribution of the cells. Replace the media with fresh MimEX™ Fibroblast Media.

    Note: *If transwell insert is not used the next day, exchange MimEX™ Fibroblast Media the morning of the procedure. Plated fibroblasts must be used within 3 days.*
14. One to two hours prior to plating Adult GI Stem Cells onto the fibroblast-coated plate, replace MimEX™ Fibroblast Media in each well with 2.0 mL of MimEX™ GI Expansion Media.

Culturing Adult GI Stem Cells

1. At least 1 day prior to plating Adult GI Stem Cells, prepare MimEX™ Irradiated Fibroblast plate as described above.
2. Warm MimEX™ GI Expansion Media to 37 °C in a water bath.
3. Warm frozen vial of Adult GI Stem Cells until just thawed and then, immediately and gently, transfer the cells to a 15 mL centrifuge tube containing at least 5.0 mL of prewarmed MimEX™ GI Expansion Media.
4. Centrifuge at 200 x g for 5 minutes.
5. Carefully remove the supernatant, re-suspend the cell pellet in 4.0 mL of MimEX™ GI Expansion Media, and mix gently by trituration.
6. Remove media from irradiated fibroblast-coated plates and add 2.0 mL of the Adult GI Stem Cell suspension to each well.
7. **Critical Step:** Evenly distribute the cells in the well using the **MimEX™ Cell Plating Procedure** as described above.
8. **Critical Step:** Incubate the plate in a **37 °C/7.5% CO_2** incubator.
9. Exchange MimEX™ GI Expansion Media every other day and monitor for colony outgrowth.
10. When colonies reach 60-80% confluency, they are ready for passaging. The colonies should not be too small nor too large and should remain isolated from one another **(Figure 1)**.

Subculturing Adult GI Stem Cells

1. At least one day prior to passaging, prepare desired number of plates with MimEX™ Irradiated Fibroblasts as described above.
2. Aspirate off the MimEX™ GI Expansion Media and add 2.0 mL of prewarmed PBS to each well, rock the plate.
3. Aspirate off the PBS and add another 1.0 mL of prewarmed PBS.
4. Dislodge mucus secreted by the Adult GI Stem Cells.
    a. Use a P1000 pipette to draw up 900 µl of PBS and gently expel it over the cells.
    b. Repeat 5-6 times. Be sure to wash the entire culture surface.
5. Remove PBS and add 1.0 mL of prewarmed 0.05% Trypsin/EDTA solution.
6. Incubate for 10 minutes at 37 °C/7.5% CO_2.
7. Check cell dissociation under the microscope. If stem cell colonies are still adhered to the culture plastic or the colonies are not thoroughly dissociated as single cells, place the plate back into the incubator and re-check every 2 minutes until the cells are dissociated.
8. Using a P1000 pipette, gently pipette the cell suspension up and down to ensure full dissociation of cells. Avoid bubbles.
9. Add 1.0 mL of MimEX™ GI Expansion Media to inhibit the trypsin, triturate gently, and transfer to a 15 mL centrifuge tube.
10. Wash the well with an additional 2.0 mL of MimEX™ GI Expansion Media and transfer to the 15 mL tube. Mix again with P1000 pipette.
11. Bring up volume to ≥ 4.0 mL with MimEX™ GI Expansion Media.
12. Centrifuge at 200 x g for 5 minutes.
13. Carefully remove the supernatant, re-suspend the cell pellet in 10 mL of MimEX™ GI Expansion Media and mix gently by trituration.

    Note: *It is recommended to passage the stem cells at a 1:10 ratio. This may need to be optimized depending on the growth of each stem cell line.*
14. Aspirate media from prepared fibroblast-coated plates and add 1.0 mL of prewarmed MimEX™ GI Expansion Media to each well.
15. Add 1.0 mL of the Adult GI Stem Cell suspension to each well.
16. **Critical Step:** Perform the **MimEX™ Cell Plating Procedure** to evenly distribute cells as described above.
17. Continue culture for 5-8 days monitoring the culture daily.
18. Exchange MimEX™ GI Expansion Media every other day.

Cryopreservation of Adult GI Stem Cells

1. Harvest cells as described above.
2. Count stem cell suspension using a hemocytometer.
3. Centrifuge at 200 x g for 5 minutes.
4. Carefully remove the supernatant and re-suspend the pellet in an appropriate amount of cryopreservation media (MimEX™ GI Expansion Media containing 20% FBS/10% DMSO).
    a. Slowly add cryopreservation media to the cell pellet with a 5.0 or 10 mL pipette.
    b. Pipette up and down 4-5 times gently to achieve even distribution of cells.

    Note: *Cryopreservation of 1 x 10^5 cells per cryovial can be thawed directly into 1 well of a 6-well plate.*
5. Fill cryovials and transfer each tube immediately for controlled slow freezing to ≤ -70 °C. The following day, transfer all tubes from ≤ -70 °C into liquid nitrogen.

DATA EXAMPLES

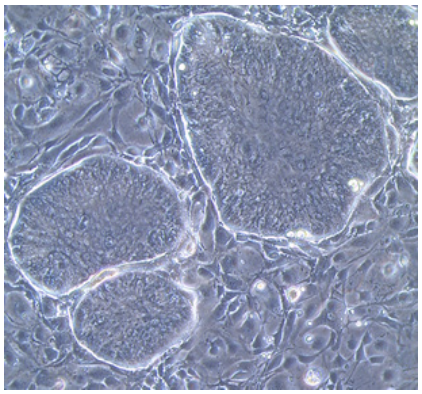

Figure 1: Intestinal Stem Cell Colony Morphology During Expansion. MimEX™ Descending Colon Stem Cells were cultured on fibroblast-coated plates in MimEX™ GI Expansion Media (R&D Systems®, Catalog # MIM003). Colony morphology was assessed using brightfield microscopy at 7 days of culture. Based on the size and distribution of the colonies, this culture is ready for passaging.

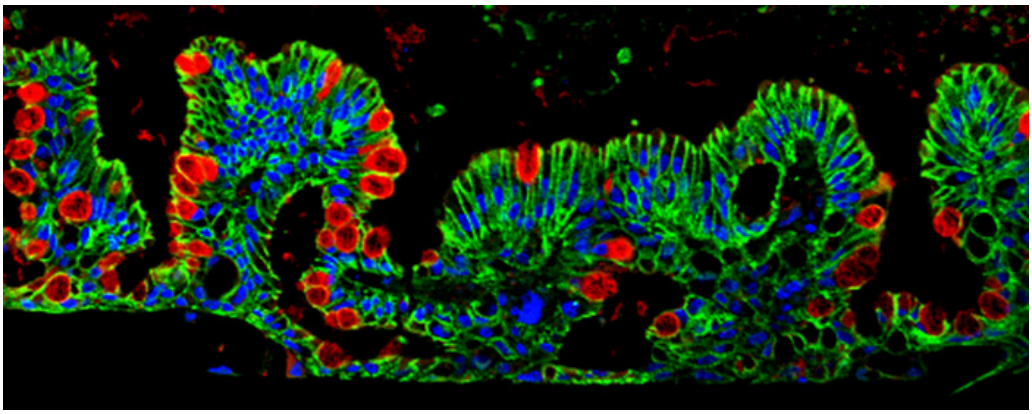

Figure 2: Human Ascending Colon Adult Stem Cells Differentiate into Ascending Colon Tissue. Adult Human GI Stem Cells from the ascending colon were expanded in MimEX™ GI Expansion Media and then differentiated in MimEX™ GI Differentiation Media (R&D Systems®, Catalog # MIM004) for 9 days under air-liquid interface conditions. To evaluate differentiation, adult stem cell-derived ascending colon tissue was immersion-fixed, paraffin-embedded, and sections stained with Mouse Anti-Human Cadherin-17 Monoclonal Antibody (green; R&D Systems®, Catalog # MAB1032) and Rabbit Anti-Human/Mouse MUC2 Antibody (red; Novus Biologicals®, Catalog # NBP1-31231). Tissue was then stained using the NorthernLights™ (NL)493-conjugated Anti-Mouse IgG Secondary Antibody (R&D Systems®, Catalog # NL009) and NL557-conjugated Anti-Rabbit IgG Secondary Antibody (R&D Systems®, Catalog # NL004) and counterstained with DAPI (blue; Tocris Bioscience®, Catalog # 5748). Cadherin-17 staining was detected in cellular membranes throughout the epithelial layer while MUC2 staining was localized specifically to goblet cells.