R&D SYSTEMS
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# MimEX™ GI Differentiation Media

**Media for the Differentiation of Adult Gastrointestinal Stem Cells**

**Catalog Number:** MIM004

**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 Differentiation Media component of this system will differentiate adult stem cell populations of the gastrointestinal tract into 3-dimensional epithelial *in vitro* tissue that mimics the regional identity and disease state of the stem cell's origin tissue (1,2).

## INTENDED USE

MimEX™ GI Differentiation Media is formulated to support the differentiation of adult epithelial stem cells derived from the gastrointestinal tract. The media has been tested for its ability to support differentiation of adult human intestinal stem cells into 3-dimensional intestinal tissue *in vitro* when cultured in an air-liquid interface.

## 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 differentiation of human adult gastrointestinal (GI) stem cells in MimEX™ GI Differentiation 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
- Transwell inserts/plates (Corning; Catalog # 3470 or equivalent).
- MimEX™ GI Expansion Media (R&D Systems®; Catalog # MIM003)
- Penicillin-Streptomycin (100X), optional
- 0.05% Trypsin/EDTA
- Sterile Phosphate-Buffered Saline (PBS) (Tocris®; Catalog # 3156)
- Mouse Feeder Removal Microbeads (Miltenyi; Catalog # 130-095-531)
- 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 Differentiation Media** - Thaw the Differentiation 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 Transwell Inserts with MimEX™ Irradiated Fibroblasts

*The following protocol is for 12 transwell inserts of a 24-well plate. If an alternate plate size is desired, adjust volumes and cell numbers accordingly.*

1. Place the 24-well plate containing transwell inserts 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 the transwell insert membrane surface with diluted Cultrex® BME.
    a. Using a P200 pipette, add 100 µL of diluted Cultrex® BME onto the membrane surface. Take care not to touch the pipette tip to the membrane surface.
    b. Rotate the plate for 2 seconds to coat the entire membrane surface.
    c. Tilt to the side and remove the diluted Cultrex® BME.
    d. Repeat for each transwell insert. Return the diluted Cultrex® BME mixture to ice every 3-4 applications to keep it cold and prevent premature polymerization.
    e. Tilt entire plate to the side and remove any excess liquid that pools to the bottom of the transwell insert.
    f. Incubate 10 min at 37 °C/7.5% $CO_2$.

## PROCEDURE *CONTINUED*

4. While the BME-coated plates are incubating, retrieve the needed vials of MimEX™ Irradiated Fibroblasts from liquid nitrogen storage. Thaw in a 37 °C water bath.
5. Aliquot the needed volume of MimEX™ Fibroblast Media (250 µL/well) into a 50 mL centrifuge tube. Each vial of MimEX™ Irradiated Fibroblasts is enough to coat 12 wells of a 24-well transwell insert plate, or the equivalent surface area.
6. Add 0.5 mL of MimEX™ Fibroblast Media to the cryovial of thawed 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.
   **Note:** *Vigorous treatment of MimEX™ Irradiated Fibroblast may impact viability and performance.*
7. Rinse the cryovial once with 0.5 mL of the fibroblast suspension, and again, slowly add it back to the 50 mL centrifuge tube dropwise while mixing by gentle swirling.
8. With forceps, carefully lift the transwell insert and add 700 µL of MimEX™ Fibroblast Media into the well of the 24-well plate (bottom). Place transwell insert back into well.
9. Add the suspension of MimEX™ Irradiated Fibroblasts onto the surface of the BME-coated transwell insert membrane (250 µL/well). Ensure there are no air bubbles in the media.
10. **Critical Step:** Evenly distribute the cells in the well using 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 in an even fashion 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).
11. **Critical step:** Incubate the plate in a **37 °C/7.5% $CO_2$** incubator overnight **without disturbing plate.**
12. Early the next morning, replace the media in the lower well and in the transwell insert 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.*
13. One to two hours prior to plating Adult GI Stem Cells onto the fibroblast-coated transwell inserts, replace the media in the bottom of the well with 250 µL of MimEX™ Expansion Media.

**Harvesting of Adult GI Stem Cells for Differentiation**

**Note:** *For each 24-well transwell insert, Adult GI Stem Cells from approximately 1 well of a 6-well plate will be needed.*

1. At least one day prior to passaging for differentiation, prepare desired number of transwell plates with MimEX™ Irradiated Fibroblasts as described above.
2. Aspirate off the MimEX™ Expansion Media from each well of expanded Adult GI Stem Cells and add 2 mL of prewarmed PBS. Rock the plate.
3. Aspirate off the PBS and add another 1 mL of prewarmed PBS.
4. Dislodge mucus secreted by the Adult GI Stem Cells.
   a. Use a P1000 pipette and 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 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 cells 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.

**Harvesting of Adult GI Stem Cells for Differentiation** *continued*

8. Using a P1000 pipette, gently pipette the cell suspension up and down to ensure full dissociation of cells. Avoid bubbles.
9. Add 1 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 mL of MimEX™ GI Expansion Media and transfer to the 15 ml tube. Mix again with P1000 pipette.
11. Bring up volume to ≥ 4 mL with MimEX™ GI Expansion Media.
12. Centrifuge at 200 x g for 5 minutes.
13. Carefully remove supernatant and re-suspend the cell pellet in 80 µL of DMEM/F12 media.
    **Note:** *If more than 2 wells are used for an experiment, pool two wells together for each fibroblast removal.*
14. During trypsinization fibroblasts are also harvested. Remove fibroblasts using the Mouse Feeder Removal Microbeads according to the manufacturer's instructions.
15. Perform a viable cell count using a hemocytometer.

**Plating for Differentiation and Tissue Formation**

1. Based on cell count, remove the required volume to yield ~450,000 cells per 24-well transwell insert needed.
2. Centrifuge the cells at 200 x g for 4 minutes.
3. Re-suspend the cells in MimEX™ GI Expansion Media at a concentration of 1.8 x $10^6$ cells/mL.
4. Into the lower well of each 24-well needed, add 700 µL of MimEX™ GI Expansion Media.
5. Remove existing media from the transell insert and add 250 µL of the Adult GI Stem Cell suspension.
6. **Critical Step:** Evenly distribute the cells using the **MimEX™ Cell Plating Procedure** described above.
7. **Critical Step:** Incubate at **37 °C/7.5% $CO_2$.**
8. Replace MimEX™ GI Expansion Media in the transwell insert and the bottom well daily.

   **Note:** *Be very careful to not touch the membrane surface of the transwell with the pipette tip when removing media. Doing so may puncture the membrane and/or dislodge adherent cells.*

   **Note:** *When cells are fully confluent and showing signs of differentiation, initiate the air-liquid interface (ALI). This will be evident as confluent Adult GI Stem Cells start to invaginate and "lines" form in the culture* ***(Figure 1).*** *Differentiation will be especially obvious around the perimeter of the transwell insert. This typically occurs 4-5 days after plating.*
9. After approximately 4-5 days, initiate the ALI by removing all media from the transwell insert. Replace the MimEX™ GI Expansion Media in the bottom well with 700 µL of MimEX™ GI Differentiation Media. Here you can use a flat coverslip forceps to grasp the transwell insert and hold it positioned at ~30-45° angle. Use a 200 µL pipette to extract the media.
10. Exchange media in the bottom well daily with 700 µL of fresh MimEX™ Differentiation Media. In the first few days, it may be necessary to remove mucus/media from the transwell insert. Here you can use a flat coverslip forceps to grasp the transwell insert and hold it positioned at ~30-45° angle using a 200 µL pipette to extract the mucus.
11. Monitor the ALI culture for maturation of differentiated GI tissue. For the small intestine and colon, the tissue will become increasingly complex with invaginations and tissue ridges that enlarge as time progresses **(Figure 1).** Tissue will be mature in approximately 8-12 days.

## DATA EXAMPLES

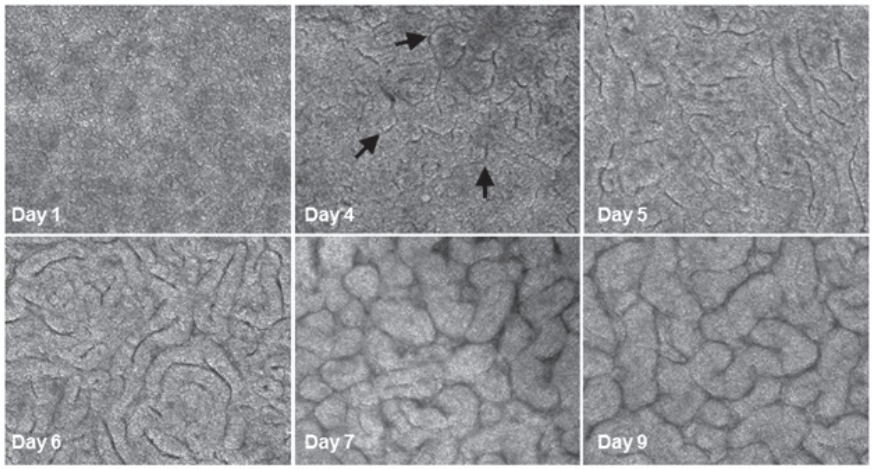


**Figure 1: Human Descending Colon Adult Stem Cell Differentiation with Air Liquid Interface (ALI) Cultures.** Adult Human GI Stem Cells from the descending colon were cultured for 9 days under ALI conditions. These phase contrast images show the progression of 3-dimensional tissue differentiation over time. On Day 4, tissue invaginations begin to form **(arrows)** indicating that the media should be switched from MimEX™ GI Expansion Media to MimEX™ GI Differentiation Media. With time, the tissue continues to differentiate forming increasingly more intricate, *in vivo*-like structures.

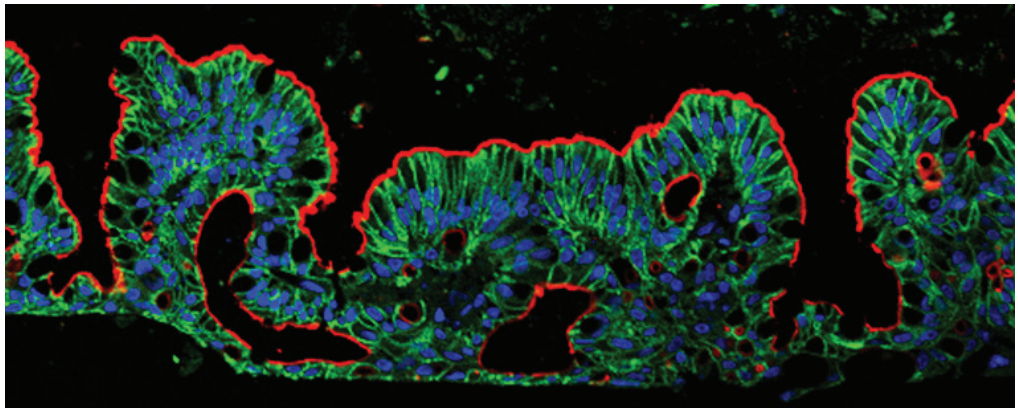

**Figure 2: Human Ascending Colon Adult Stem Cells Differentiate into Ascending Colon Tissue.** Adult Human GI Stem Cells from the ascending colon were expanded in MimEX™ GI Expansion Media and then differentiated in MimEX™ GI Differentiation Media for 9 days under air-liquid interface conditions. To 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/Rat/Bovine Villin 1 Antibody (red; Novus Biologicals®, Catalog # NBP1-32841). 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 Villin 1 staining was localized specifically to the apical surface of the polarized epithelial layer.