MimEX™

GI Starter Kit

Catalog Number MIM002

Reagents for the expansion and differentiation of adult gastrointestinal epithelial stem cells.

This package insert must be read in its entirety before using this product.
For laboratory research use only. Not for diagnostic use.
The safety and efficacy of this product in diagnostic or
other clinical uses has not been established.

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USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Adult stem cell populations are found in most tissues of the human body and provide a long-lived source of self-renewing cells for tissue homeostasis under normal conditions and regeneration in disease or injury. In the adult, tissue specific stem cells can be unipotent or multipotent depending on the nature of the tissue, but they all exhibit an epigenetic memory conferring tissue specificity and regional identity. Moreover, adult stem cells may hold the keys to understanding aging, disease progression, and the development and spread of cancer.

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 tissues *in vitro* and provide a valuable tool for biomarker discovery, disease modeling, drug screening, and developmental biology studies. The MimEX™ GI Starter Kit will expand adult stem cell populations of the gastrointestinal tract under clonal conditions while maintaining regional identity, genome stability, and disease state. It will also differentiate gastrointestinal adult stem cells into 3-dimensional epithelial *in vitro* tissue that mimics the regional cytoarchitechture or disease state of the stem cell's origin tissue (1,2).

PRINCIPLE OF THE ASSAY

The MimEX™ GI Starter Kit contains specially formulated reagents and plates for the expansion and differentiation of adult gastrointestinal epithelial stem cells. The quantity of each component is sufficient to expand stem cells to generate enough cells for differentiation into 12 transwell inserts in a 24-well tissue culture plate.

TECHNICAL HINTS & LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix or substitute reagents with those from other lots or sources.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

PRECAUTION

The acute and chronic effects of overexposure to reagents of this kit are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling kit reagents.

MATERIALS PROVIDED & STORAGE CONDITIONS

Note: *The components for this kit require different storage/shipping temperatures and will arrive in separate packaging.*

PART	PART #	DESCRIPTION	STORAGE OF UNOPENED MATERIAL	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
MimEX™ GI Expansion Media	390617	250 mL of ready to use media.	Store at ≤ -20 °C in a manual defrost freezer.	Store at 2-8 °C for up to 2 weeks or aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 3 months.* Avoid repeated freeze-thaw cycles.
MimEX™ GI Differentiation Media	390618	100 mL of ready to use media.	Store at ≤ -20 °C in a manual defrost freezer.	
MimEX™ Irradiated Fibroblasts	390614	12 vials each containing enough cells to coat 2 wells of a 6-well plate or the equivalent surface area.	Store in liquid nitrogen.	Must be used immediately upon thaw.
MimEX™ Fibroblast Media	390610	2 vials each containing 50 mL of ready to use media.	Store under sterile conditions at ≤ -20 °C in a manual defrost freezer.*	Store at 2-8 °C for up to 2 weeks or aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 3 months.* Avoid repeated freeze-thaw cycles.
Stem Cell Qualified RGF BME, Pathclear®	894860	1 frozen vial containing 1.5 mL of concentrated solution.	Store at ≤ -70 °C in a manual defrost freezer.	Store at 2-8 °C on ice for up to 1 week or aliquot and store at ≤ -70 °C for up to 3 months.* Avoid repeated freeze-thaw cycles
Transwell Insert, 24-well plate	608113	1 24-well plate containing 12 transwell inserts.	Store at room temperature until use.	Store at room temperature until use.

*Provided this is within the expiration of the kit.

OTHER SUPPLIES REQUIRED

- Adult GI Stem Cells (purchased directly from R&D Systems or isolated from tissue using the MimEX™ Tissue Processing Kit (R&D Systems®, Catalog # MIM001))
- DMEM/F12
- Penicillin-Streptomycin (100X), optional
- 0.05% Trypsin/EDTA
- 6-well tissue culture treated plates
- 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 & MATERIAL 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.

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.

MimEX™ Fibroblast Media - Thaw the MimEX™ Fibroblast 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 FOR EXPANSION OF HUMAN ADULT GASTROTINTESTINAL STEM CELLS

The protocol below describes the expansion of human adult gastrointestinal (GI) stem cells using the reagents provided in the MimEX™ GI Starter Kit.

Note: *This protocol must be read in its entirety before using this product.*

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 of 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 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.
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.

Preparing Plates with MimEX™ Irradiated Fibroblasts *continued*

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 mL of MimEX™ 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 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 2.0 mL of MimEX™ GI Expansion Media, and mix gently by trituration.
6. Remove media from irradiated fibroblast-coated plates and add 2 mL of the Adult GI Stem Cell suspension to one 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 a 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. Add 1.0 mL of prewarmed MimEX™ GI Expansion Media.
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 Adult GI Stem 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.

PROCEDURE FOR DIFFERENTIATION OF HUMAN ADULT GASTROTINTESTINAL STEM CELLS

The protocol below describes the differentiation of human adult gastrointestinal (GI) stem cells using the reagents provided in the MimEX™ GI Starter Kit.

Note: This protocol must be read in its entirety before using this product.

Preparing Transwell Inserts with MimEX™ Irradiated Fibroblasts

The following protocol is to plate one vial of MimEX™ Irradiated Fibroblasts into 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 Cultrex® BME that pools to the bottom of the transwell insert.
    f. Incubate 10 min at 37 °C/7.5% CO_2.

Preparing Transwell Inserts with MimEX™ Irradiated Fibroblasts *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 transwell inserts 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 vigorously.
Note: *Vigorous treatment of MimEX™ Irradiated Fibroblasts 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 the 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 lower well (700 µL) and transwell insert (250 µL) of the well with 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 insert 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 2.0 mL of prewarmed PBS. 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 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 1.0 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 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 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 1.8×10^6 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 transwell 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 3).*** *Differentiation will be especially obvious around the perimeter of the transwell insert. This typically occurs 4-5 days after plating.*
9. After 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. 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 3).** Tissue will be mature in approximately 8-12 days.

Fixing & Staining Procedures

Note: *Prior to performing either of the fixation and staining procedures below, remove mucus from the surface of the GI tissue using the methods described above.*

Paraffin Embedding of MimEX™-generated GI Tissue - Tissues generated in the transwell inserts using MimEX™ GI Reagents can be prepared for paraffin embedding by immersion-fixation in 4% paraformaldehyde at room temperature for 4-8 hours. Paraffin-embedded tissue can then be processed for immunohistochemical staining using standard methods. For a more detailed protocol, please visit: www.rndsystems.com/protocols

Whole Mount Preparation of MimEX™-generated GI Tissue - Tissues generated in transwell inserts using MimEX™ Reagents can be prepared for whole mount analysis by adding 4% paraformaldehyde to the top and bottom chamber of the transwell insert. Incubate overnight at 2-8 °C, rinse with PBS, and then process for immunohistochemical staining using standard methods.

DATA EXAMPLES

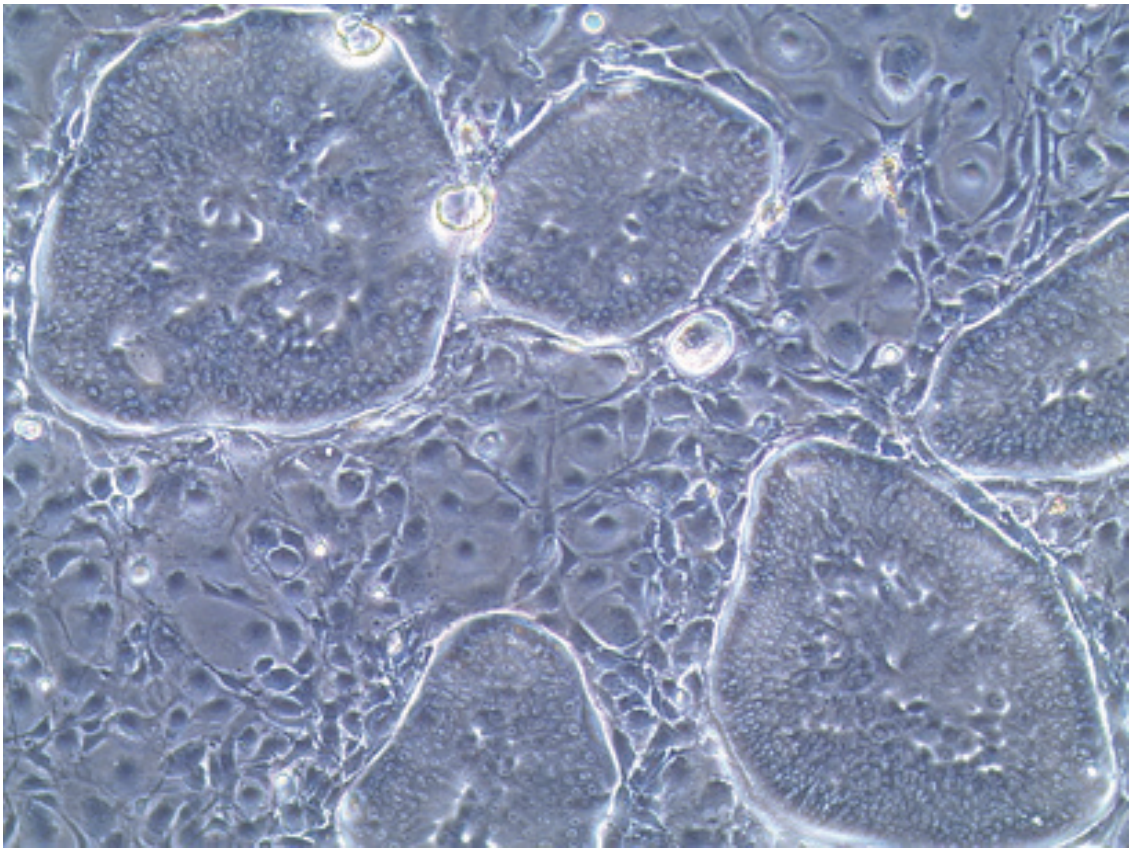

Figure 1: Human Ileum Adult Stem Cell Colonies on Irradiated Fibroblasts. Adult stem cells were isolated from human ileum tissue using the MimEX™ Tissue Processing Kit (R&D Systems®, Catalog # MIM001). Ileum adult stem cells were cultured for 7 days in MimEX™ GI Expansion Media and MimEX™ Irradiated Fibroblasts. This phase contrast image shows colonies of ileum adult stem cells. These colonies show good distribution and are the ideal size for passaging.

DATA EXAMPLES *CONTINUED*

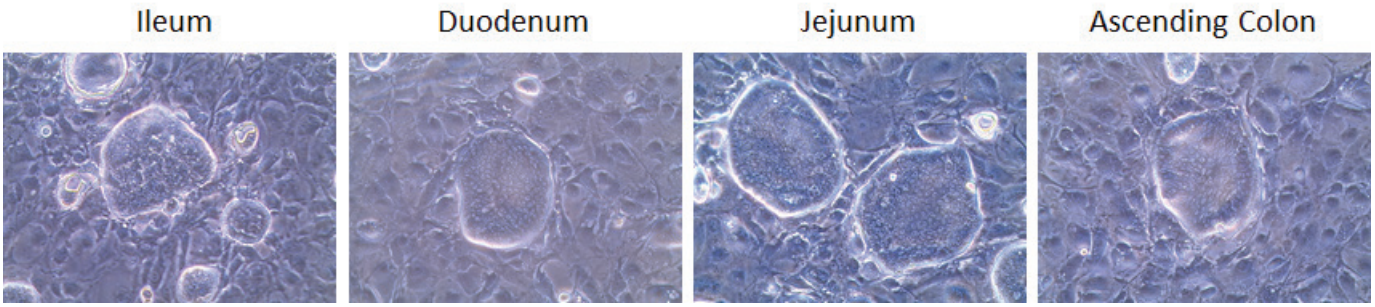

Figure 2: Isolation of Adult Stem Cells from Specific Regions of the Gastrointestinal Tract. Tissue biopsies from the human ileum, duodenum, jejunum, and ascending colon were procured and processed using the MimEX™ Tissue Processing Kit (R&D Systems®, Catalog # MIM001). Images show adult stem cell colonies isolated from the region specific gastrointestinal tissue following expansion and passaging using MimEX™ GI Expansion Media and MimEX™ Irradiated Fibroblasts, which are included in the this kit.

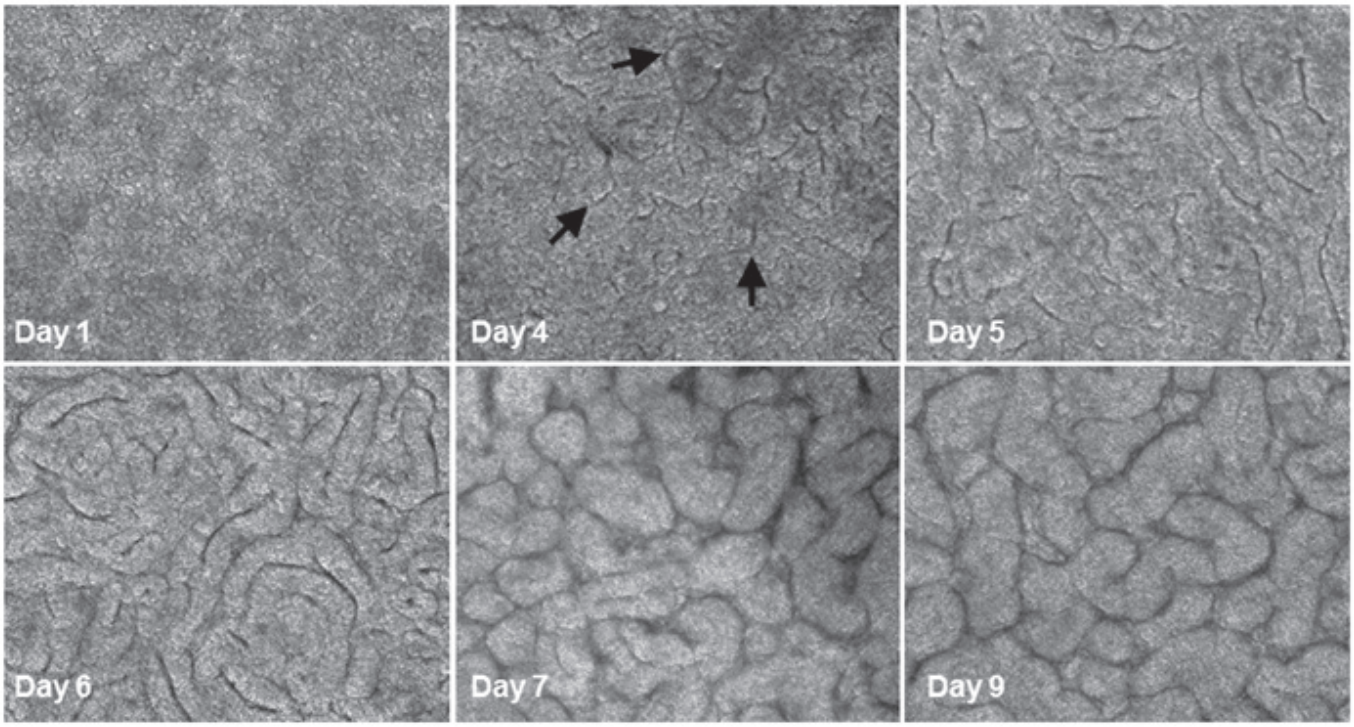

Figure 3: Human Descending Colon Adult Stem Cell Differentiation with Air-Liquid Interface (ALI) Cultures. Adult Human GI Stem Cells from the descending colon were cultured for 9 days under ALI conditions. These phase contrast images show the progression of 3-dimensional tissue differentiation over time.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

DATA EXAMPLES *CONTINUED*

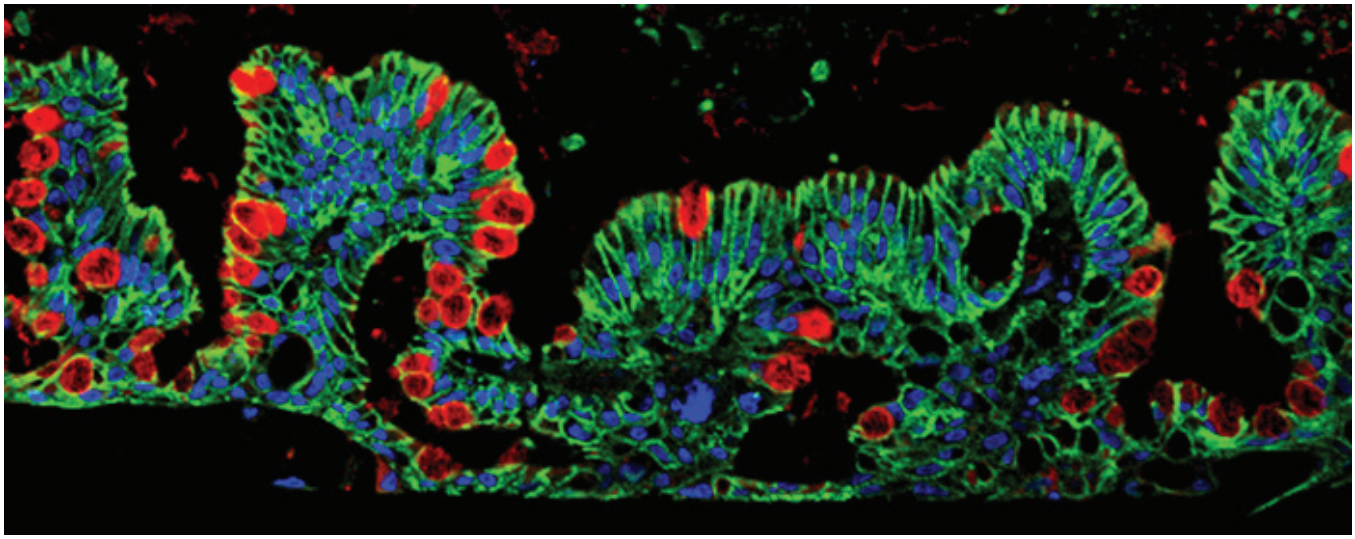

Figure 4: Human Ascending Colon Adult Stem Cells Differentiate into Ascending Colon Tissue. Adult Human GI Stem Cells from the ascending colon were cultured in MimEX™ GI Media for 9 days under air-liquid interface conditions. To evaluate differentiation, MimEX™ adult stem cell-derived ascending colon 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®; Catalog # 5748). Cadherin-17 staining was detected in cellular membranes throughout the epithelial layer while MUC2 staining was localized specifically to goblet cells.

REFERENCES

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

NOTES

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