R&D SYSTEMS
a bio-techne brand

# MimEX™ Irradiated Fibroblast Kit

**Feeder Cells for the Culture of Adult Epithelial Stem Cells**

**Catalog Number:** MIM005

## PRODUCT DESCRIPTION

The MimEX™ Tissue Model System allows for the expansion and differentiation of ground-state, adult stem cell populations. 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™ Irradiated Fibroblast Kit component of this system supports the expansion and differentiation of adult epithelial stem cell populations under clonal conditions while maintaining regional identity, genome stability, and disease state (1,2).

## INTENDED USE

The MimEX™ Irradiated Fibroblast Kit is formulated to support the expansion and differentiation of adult epithelial stem cells. The cells and media have been tested for their ability to support expansion of adult intestinal stem cells *in vitro*.

## MATERIALS PROVIDED & STORAGE

| PART | PART # | DESCRIPTION | STORAGE OF UNOPENED MATERIAL | STORAGE OF OPENED/DILUTED MATERIAL |
|---|---|---|---|---|
| **MimEX™ Irradiated Fibroblasts** | 390614 | 6 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 | 50 mL of 1X media. | Store under sterile conditions at ≤ -20 °C in a manual defrost freezer.* | Store at 2-8 °C for up to 1 month, or aliquot and store ≤ -20 °C in a manual defrost freezer.* |
| **Cultrex® Stem Cell Qualified RGF BME, 1 mL** | 896212 | 1 mL of frozen reduced growth factor basement membrane extract. | Store at ≤ -70 °C.* | Store at 2-8 °C on ice for up to 1 week, or aliquot and store ≤ -70 °C for up to 3 months.* Avoid repeated freeze-thaw cycles. |

* Provided this is within the expiration date of the kit.

## 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.





bio-techne.com
info@bio-techne.com
techsupport@bio-techne.com

North America
TEL 800 343 7475

Europe • Middle East • Africa
TEL +44 (0)1235 529449

China
info.cn@bio-techne.com
TEL +86 (21) 52380373

Rest of World
bio-techne.com/find-us/distributors
TEL +1 612 379 2956

## PROCEDURE FOR THE PLATING OF IRRADIATED FIBROBLASTS

The protocol below describes the plating of MimEX™ Irradiated Fibroblasts for the expansion or differentiation of epithelial stem cells.

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

## OTHER MATERIALS REQUIRED

- DMEM/F12.
- Penicillin-Streptomycin (100X), optional.
- 6-well tissue culture treated plates.
- 50 mL centrifuge tubes.
- Serological pipettes.
- Pipette and pipette tips.
- 37 °C and 7.5% $CO_2$ humidified incubator .
- Inverted Microscope.
- Water bath.

## REAGENT PREPARATION

**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

### Plating MimEX™ Irradiated Fibroblasts for Stem Cell Expansion

*The following protocol is to plate one vial of MimEX™ Irradiated Fibroblasts into 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 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 (2.0 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.

## PROCEDURE *CONTINUED*

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™ Expansion Media.

**Plating MimEX™ Irradiated Fibroblasts for Stem Cell Differentiation**

*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% $CO_2$.
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.

**Plating MimEX™ Irradiated Fibroblasts for Stem Cell Differentiation** *continued*

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 **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. **Critical Step:** 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.

## DATA EXAMPLES

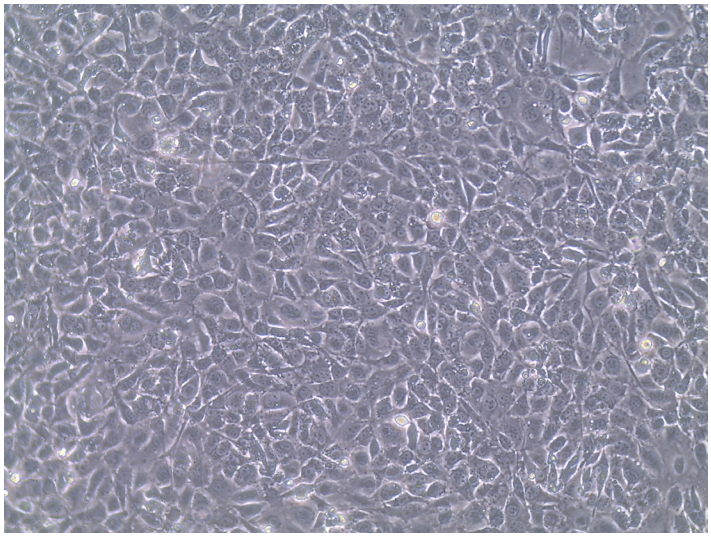

**Figure 1: Irradiated Fibroblast Plating Density and Distribution the Day After Plating for Stem Cell Expansion.** MimEX™ Irradiated Fibroblasts were plated and cultured for one day in MimEX™ Fibroblast Media. Seeding density was evaluated with phase contrast imaging which shows a dense monolayer of cells that are evenly distributed on the culture surface.