Mouse Embryonic Fibroblast (MEF) Conditioned Media

Catalog Number: AR005
Volume: 100 mL

PRODUCT DESCRIPTION

Mouse Embryonic Fibroblast (MEF) Conditioned Media was prepared according to the protocol published by Xu, C.*et al.* (1). Serum-free media [80% Knockout™ DMEM, 20% Knockout™ serum replacement, 1% MEM non-essential amino acids, 2 mM GlutaMAX™, 0.1 mM β-mercaptoethanol, 4 ng/mL basic Fibroblast Growth Factor (FGF basic)] was conditioned by γ-irradiated CF-1 fibroblasts at 37 °C for 24 hours.

INTENDED USE

MEF conditioned media has been shown to support human pluripotent stem cell (PSC) growth and to maintain PSC pluripotency in the presence of FGF basic and Laminin (1, 2). It can be used as a substitute for MEF feeder layers in human PSC culture. MEF Conditioned Media has been tested for its ability to support Oct-3/4$^+$, SSEA-4$^+$ human PSC growth *in vitro*.

STABILITY & STORAGE

Upon receipt, store the conditioned media at ≤ -20° C in a manual defrost freezer. When ready to use, thaw the solution overnight at 2-8 °C in the dark. Aliquot and store the unused portions at ≤ -20 °C in a manual defrost freezer. Long-term storage at 2-8 °C is not recommended. Avoid repeated freeze-thaw cycles and do not use past the expiration date. The color of this product may vary. Color variation does not affect performance.

ENDOTOXIN LEVEL

Acceptable endotoxin level of < 0.1 EU/mL.

MYCOPLASMA TEST

Mycoplasma negative as tested by the MycoProbe™ Mycoplasma Detection Kit (R&D Systems, Catalog # CUL001B).

PRECAUTION

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

REFERENCES

1. Xu, C. *et al.* (2001) Nat. Biotechnol. **19**:971.
2. Rosler, E.S. *et al.* (2004) Dev. Dyn. **229**:259.
3. Carpenter, M.K. *et al.* (2004) Dev. Dyn. **229**:243.

CULTURING BG01V HUMAN EMBRYONIC STEM CELLS IN MOUSE EMBRYONIC FIBROBLAST (MEF) CONDITIONED MEDIA

The protocol below has been used with the BG01V human embryonic stem cell line. Please note that the use of other PSC lines may require modifications of this protocol. Optimal culture conditions for each pluripotent stem cell line must be determined by the investigator.

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

OTHER SUPPLIES REQUIRED

Reagents

- Recombinant Human FGF basic (R&D Systems, Catalog # 233-FB) or tissue culture grade FGF basic (R&D Systems, Catalog # 4114-TC)
- Accutase® (Innovative Cell Technologies, Catalog # AT104 or equivalent)
- Cultrex® PathClear® Stem Cell Qualified Reduced Growth Factor Basement Membrane Extract (BME) (R&D Systems, Catalog # 3434-001-02)
- DMEM/F12

Materials

- BG01V human embryonic stem cells (ATCC, Catalog # SCRC-2002) or equivalent
- 60 or 100 mm tissue culture plates
- 15 mL centrifuge tubes
- Pipettes and pipette tips

Equipment

- 37 °C, 5% CO_2 humidified incubator
- Centrifuge (low speed clinical or equivalent)
- Hemocytometer
- Microscope

REAGENT PREPARATION

Complete MEF Conditioned Media - Prepare complete media by adding FGF basic to the Conditioned Media at a concentration of 4 ng/mL.

PREPARATION OF CULTREX BME-COATED PLATES

1. Thaw Cultrex BME at 2-8 °C overnight.
2. Aliquot thawed Cultrex BME into pre-cooled tubes and store at ≤ -20 °C.
3. Thaw the aliquot on ice or at 2-8 °C overnight.
4. Dilute Cultrex BME 1:40 in DMEM/F12. This can be stored at 2-8 °C for up to 2 weeks.
5. Coat the desired number of plates with diluted Cultrex BME (approximately 2.5 mL/60 mm plate) and incubate for 1-2 hours at room temperature.
6. Remove the Cultrex BME solution immediately prior to plating the cells.

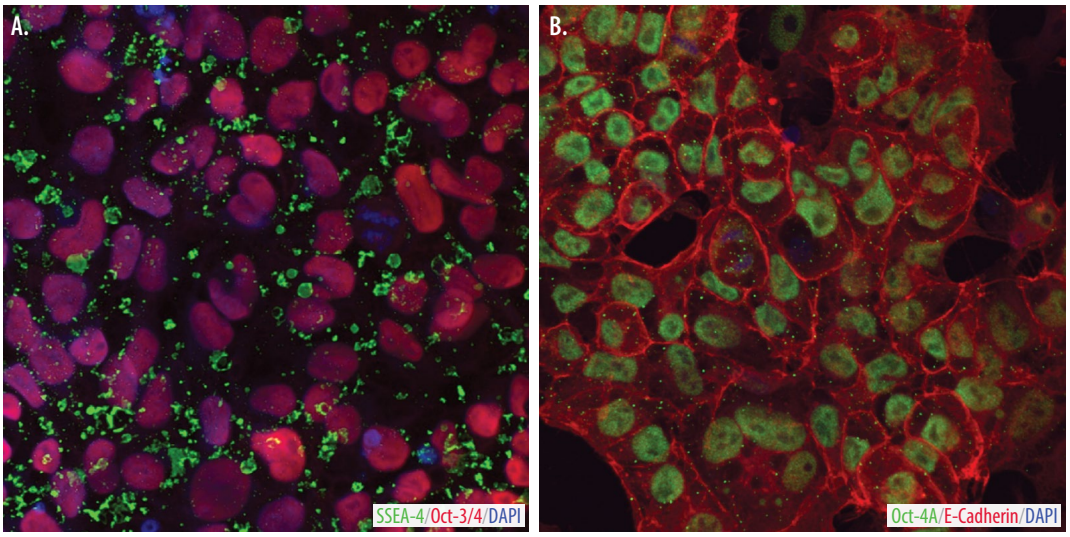
PREPARATION AND PLATING OF BG01V HUMAN EMBRYONIC STEM CELLS

1. Warm the MEF Conditioned Media to 37 °C.
2. Warm the vial of BG01V human embryonic 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 pre-warmed MEF Conditioned Media. Rinse the cryovial with an additional 1 mL of media to ensure that all of the cells have been removed.
3. Centrifuge at 200 x g for 4 minutes.
4. Remove the supernatant and resuspend the pellet in an appropriate amount of Complete MEF Media.
5. Add the BG01V human embryonic stem cell suspension to the Cultrex BME-coated plate.
6. Culture the cells in a 37 °C and 5% CO_2 incubator. Change the media daily and monitor the cells. Passage the cells at the desired confluency.

PASSAGING BG01V HUMAN EMBRYONIC STEM CELLS

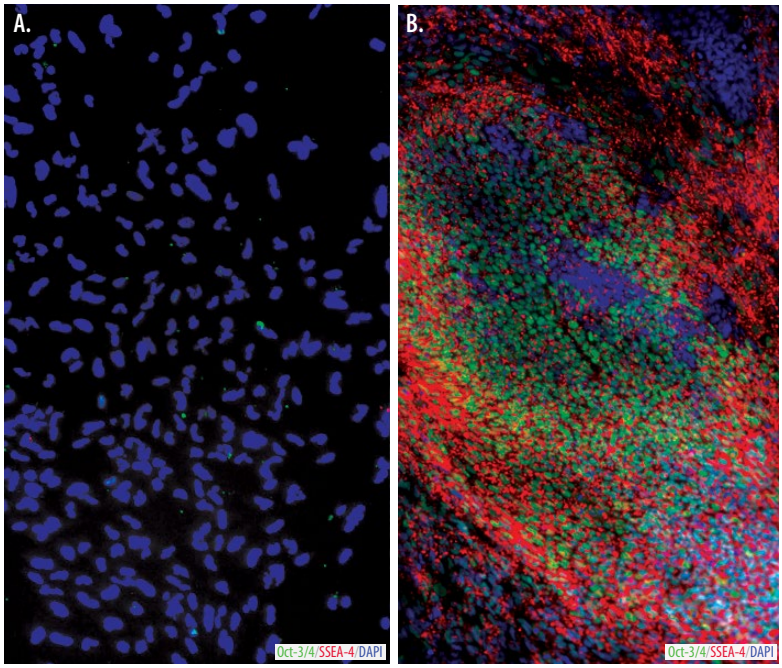
1. Coat the desired number of plates with Cultrex BME 1-2 hours prior to passaging the cells, as described above.
2. Warm the MEF Conditioned Media to 37 °C.
3. Remove the MEF Conditioned Media from the cells. Add 1 mL of Accutase to each 60 mm plate. Incubate at room temperature for approximately 5 minutes or until the cells begin to detach from the plate.
4. Pipette the Accutase gently over the plate until all of the cells have been detached.
5. Pipette the cell suspension up and down to break up large cell clumps.
6. Transfer the cell suspension to a 15 mL centrifuge tube containing 5 mL of MEF Conditioned Media and centrifuge at 200 x g for 4 minutes.
7. Resuspend the pellet in Complete MEF Conditioned Media and count the viable cells using a hemocytometer.
8. Plate the desired number of cells (approximately 1.0 x 10^6 cells/60 mm plate) on the Cultrex BME-coated plate in Complete MEF Conditioned Media.
9. Change the media daily. Monitor the cells for the desired confluency.

DATA EXAMPLES



Induced Pluripotent Stem Cells Grown in MEF Conditioned Media Express Pluripotency Stem Cell Markers SSEA-4, Oct-3/4, Oct-4A, and E-Cadherin. Human iPS2 stem cells were cultured in MEF Conditioned Media supplemented with 4 ng/mL Recombinant Human FGF basic (R&D Systems, Catalog # 233-FB or Catalog # 4114-TC). **A.** Expression of SSEA-4 was detected using Mouse Anti-Human/Mouse SSEA-4 Monoclonal Antibody (R&D Systems, Catalog # MAB1435) followed by NorthernLights™ (NL) 493-conjugated Donkey Anti-Mouse Secondary Antibody (R&D Systems, Catalog # NL009; green). Expression of Oct-3/4 was detected using Goat Anti-Human Oct-3/4 Antigen Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF1759) followed by NL557-conjugated Donkey Anti-Goat Secondary Antibody (R&D Systems, Catalog NL001; red). The nuclei were counterstained with DAPI. **B.** Expression of Oct-4A was detected using Mouse Anti-Human Oct-4A Monoclonal Antibody (R&D Systems, Catalog # MAB17591) followed by NL493-conjugated Donkey Anti-Mouse Polyclonal Antibody (R&D Systems, Catalog # NL009; green). Expression of E-Cadherin was detected using Goat Anti-Human E-Cadherin Antigen Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF648) followed by NL557-conjugated Donkey Anti-Goat Secondary Antibody (R&D Systems, Catalog # NL001; red). The nuclei were counterstained with DAPI.

DATA EXAMPLES



Human Embryonic Stem Cells Cultured in MEF Conditioned Media Express Pluripotency Markers SSEA-4 and Oct-3/4. Human embryonic stem cells were cultured in **A.** basal media supplemented with Recombinant Human FGF basic (R&D Systems, Catalog # 233-FB) or **B.** in MEF Conditioned Media supplemented with Recombinant Human FGF basic. SSEA-4 and Oct-3/4 were detected using Mouse Anti-Human/Mouse SSEA-4 Monoclonal Antibody (R&D Systems, Catalog # MAB1435; red) and Goat Anti-Human Oct-3/4 Antigen Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF1759; green). The nuclei were counterstained with DAPI (blue). *Image courtesy of Dr. Frank Soldner of the National Institutes of Health.*

BG01V cells are licensed from ViaCyte, Inc.