Corning® BioCoat™ Matrigel® Matrix 6-well Plates for Embryonic Stem (ES) Cell Culture

Catalog Number 354671

Guidelines for Use

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CORNING

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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INTENDED USE

Corning® Matrigel® Matrix has been used extensively as a substrate for culturing human embryonic stem (ES) cells with various conditioned or defined media.[1-10]

Corning BioCoat Matrigel Matrix 6-well Plates for ES Cell Culture provides an optimized surface for culturing human ES cells while maintaining their ability for self-renewal and pluripotency. It is recommended that cells on Corning BioCoat Matrigel Matrix 6-well plates for ES cell culture be cultured using mouse embryonic fibroblast (MEF) feeder-conditioned media (MEF-CM) supplemented with 8 ng/mL Corning basic fibroblast growth factor (bFGF; Cat #354060).

This product is **FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

MATERIALS PROVIDED

Catalog No. 354671

- Corning BioCoat Matrigel Matrix 6-well Plates for ES Cell Culture. Comprised of 5 lidded Falcon® 6 well plates coated with Corning Matrigel Matrix.

QUALITY CONTROL

- Each lot of Corning BioCoat Matrigel Matrix 6-well Plate for ES cell culture is tested for the amount of functional proteins coated onto the surface of each well to ensure lot-to-lot consistency.
- Functional performance has been validated by culturing undifferentiated human ES cells (H9, H14, or H1 from WiCell Institute) in combination with MEF-CM supplemented with 8 ng/mL Corning bFGF (this is not a release criteria).
- Tested and found negative for bacteria and fungi.

MATERIALS REQUIRED BUT NOT SUPPLIED

- MEF feeder cells isolated from *CF-1* mouse strain (inactivated through irradiation or mitomycin C-treatment; ATCC Cat # SCRC 1040.1 or SCRC1040.2 respectively).
- Dulbecco's Phosphate Buffered Saline (DPBS)
- Gelatin
- Fibroblast culture media components
    - DMEM media (Invitrogen, Cat # 11965-092)
    - L-Glutamine 200 mM
    - Non-essential Amino Acids
    - Fetal Bovine Serum (FBS) – Heat inactivated by heating to 56°C for 30 minutes.

- human ES cell culture and media components
    - DMEM/F12 medium (Invitrogen, Cat # 113330-032)
    - KnockOut™ Serum Replacement (Invitrogen, Cat # 108280-028)
    - MEM-Non-essential amino acids (10mM)
    - L-Glutamine (200mM)
    - 2-Mercaptoethanol

RELATED CORNING PRODUCTS, NOT SUPPLIED

- Corning® Dispase, Cat # 354235 (may be used as an alternative to collagenase Type IV for human ES cell dissociation)
- Corning Human basic FGF, Cat # 354060
- Falcon® 6-well Tissue Culture-treated plate, Cat # 353046
- Corning Delipidized BSA, Cat # 354331

PRECAUTIONS

a.) **Storage: Corning BioCoat™ Matrigel® Matrix 6-well plates for ES cell culture should be stored at -20°C in the original packaging in order to guarantee shelf life. All plates should be used once the package is opened and thawed. DO NOT THAW AND RE-FREEZE PLATES. Aseptically remove desired number of plates from packet, re-seal the remaining plates in packet with tape and store at -20°C PROMPTLY (within 5 minutes). Use the re-packaged plates within two weeks.**

b.) **All procedures should be performed under aseptic conditions**.

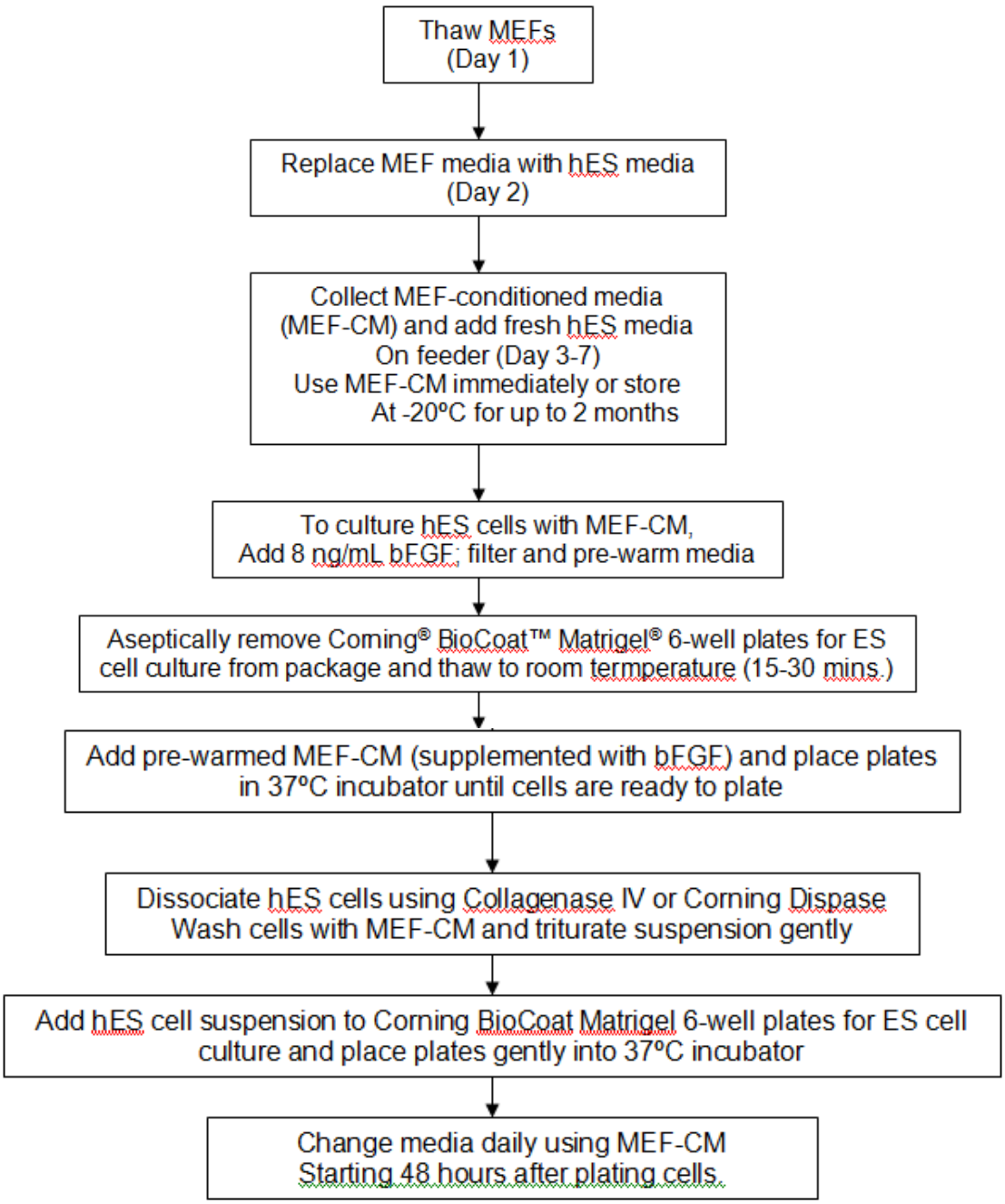
California Proposition 65 Notice

WARNING:	This product contains a chemical known to the state of California to cause cancer.
Component:	**Chloroform**

PROCEDURE FOR USE

The following procedure is optimized for culturing human ES cells initially grown on MEF feeder layers or on Corning BioCoat Matrigel 6-well plates for ES cell culture. Results may vary depending upon the cell line used, the type of feeder cells used and the specific conditions and health of the cells to be plated (e.g. media, state of differentiation, dissociation technique, etc.). You should optimize conditions for your own system.

Figure1. Steps involved in culturing human ES cells on Corning® BioCoat™ Matrigel® 6-well plates for ES cell culture†.



†NOTE: Corning BioCoat Matrigel 6-well plates for ES cell culture (Cat# 354671) are specifically developed for culturing ES cells and are distinct from other Corning BioCoat Matrigel Matrix 6-well plates (Cat# 354432 & 354603).

1.0 Preparation of solutions

1.1 Preparation of 0.1% Gelatin Solution (for 500 ml)

1.1.1 Add 0.5 g of gelatin to 500 mL of tissue culture grade endotoxin-free ddH_2O. Gelatin will not dissolve completely in water. Autoclave to dissolve gelatin and sterilize the solution. Once solution has cooled, store at 4°C.

1.2 Preparation of human bFGF

1.2.1 Dissolve 10 μg of bFGF in 1 mL of PBS containing 0.2% BSA. Aliquot and store at -20°C.

1.3 Preparation Collagenase IV (200 units/mL)

1.3.1 Dissolve 20,000 units of collagenase IV in 100 mL of DMEM (final concentration of 1 mg/mL). Filter sterilize, aliquot in 5-10 mL/tubes and store at -20°C.
NOTE: Corning® Dispase can be substituted for collagenase IV (recommended concentration for Corning Dispase is 1.0 to 1.25 units/mL DMEM/F-12).

1.4 Preparation of MEF medium (500 mL)

1.4.1 To 440 mL DMEM medium, add 5 mL of non-essential amino acids, 5 mL of L-glutamine, and 50 mL of heat-inactivated FBS. Filter, and store at 4°C.

1.5 Preparation of human ES cell medium (100 mL)

1.5.1 To 80 mL of DMEM/F12 medium, add 20 mL of KnockOut™ serum replacement (20%), 1 mL of non-essential amino acids, 0.5 mL of L-glutamine and β-mercaptoethanol (0.1 mM final concentration) and 100 μL bFGF (10 ng/mL). Filter, and store at 4°C for up to 2 weeks.

1.6 Preparation of Embryoid Body Formation medium (100 mL)

1.6.1 To 78 mL of DMEM/F12, add 20 mL FBS (not heat-inactivated), 1 mL non-essential amino acids, 1 mL L-glutamine and β-mercaptoethanol (0.1 mM final concentration). Filter and store at 4°C for up to 1 month.

2.0 Generating MEF-conditioned media (MEF-CM)

2.1 Thawing inactivated MEF feeder cells

2.1.1 Gelatin coat plates or flask by adding 2 mL of 0.1% gelatin solution to each well of the 6 well plate (10 mL for T75 and 20 mL for T175 flasks) and incubate at room temperature for 1 hour or overnight.

2.1.2 Aspirate off gelatin immediately prior to plating MEF feeder cells.

2.1.3 Thaw a vial of MEF by immersing it in a 37°C water bath without submerging the vial below the O-ring on the cap. Spray the vial with 70% ethanol and wipe it dry with a clean Kimwipe®.

2.1.4 Transfer contents of the vial into a sterile 15 mL conical tube in a tissue culture hood where the rest of the procedures will be performed aseptically. Add 10 mL of pre-warmed MEF media drop-wise, swirling the contents gently as you add.

2.1.5 Centrifuge at 1000 rpm for 5 minutes and discard supernatant. Re-suspend cell pellet in MEF media (0.75×10^5 cells/mL) and add 2.5 mL of cell suspension to each gelatinized well of 6 well plates (or 20 mL/T75, 45 mL/T175). Make sure cells are spread evenly in the wells or flask and incubate overnight in 5% CO_2 and humidified air at 37°C.

2.2 Collect MEF-CM media for culturing human ES cells

2.2.1 After MEF feeders have been incubated overnight, aspirate off MEF medium and wash once with 2 mL of PBS.

2.2.2 Add 3 ml of human ES cell medium supplemented with 10 ng/mL of bFGF to each well (24ml/T-75, 50ml/T-175) and incubate overnight.

2.2.3 Following 24 hour incubation with MEF feeder cells, collect MEF-CM, add 8 ng/mL of bFGF, filter and use immediately for culturing human ES cell cells on Corning® BioCoat™ Matrigel® Matrix 6-well plates for ES cell culture.
NOTE: MEF-CM can be stored for up to 7 days at 4°C and up to 2 months at -20°C. Supplement stored MEF-CM with 8 ng/mL bFGF and filter immediately prior to use.

3.0 Dissociation and plating of human ES cells on Corning BioCoat Matrigel 6-

well plates for ES cell culture

3.1 Add pre-warmed MEF-CM (2mL/well) supplemented with 8 ng/mL bFGF to Corning BioCoat Matrigel 6-well plates for ES cell culture and place the plates in a 37°C incubator with 5% CO_2 and humidified air.

3.2 Aspirate medium from wells containing human ES cells to be dissociated (cultured on MEF feeders or Corning BioCoat Matrigel 6-well plates for ES cell culture). Wash once briefly with 2 mL PBS and aspirate.

3.3 Treat human ES cells to be dissociated with 200 units/mL of collagenase IV or dispase (1 – 1.25 units/mL) for 6-8 minutes at 37°C, or until the edges of the colony start to curl up when observed under the microscope.

3.4 Aspirate collagenase IV or dispase and wash once gently with 2 mL of MEF-CM. After wash, add another 2 mL of MEF-CM per well.

3.5 Practicing proper aseptic technique, and using a sterile fine pipette tip, gently break-up and scrape the colonies using small circular motions. Start at the outside edge of the colony and work your way towards the middle. Try to cut as many small pieces as possible. This is easiest to do while viewing the process using a phase contrast microscope (2x or 4x objective).

3.6 Gently triturate cell clumps a few times by pipetting up and down (do not make a single cell suspension). Distribute 0.5 mL of cell suspension to each well on Corning® BioCoat™ Matrigel® 6-well plates for ES cell culture containing pre-warmed MEF-CM and place plates in incubator. As a rough guideline, split cells at a 1:3 to 1:6 ratio.

NOTE: it is important not to swirl contents of plate as this will result in colonies crowding in the center of the plate. Gently rock the plate side to side and back and forth just prior to placing into incubator to evenly distribute the cell clumps on the plate surface.

4.0 Maintenance of human ES cells

NOTE: Following dissociation of human ES cells, the cultures should be left undisturbed for the next day (Day 2). Start changing media from Day 3 after plating cells.

4.1 Aspirate spent media from Corning BioCoat Matrigel 6-well plates for ES cell culture and add 2.5 to 3 mL or pre-warmed and sterile-filtered MEF-CM supplemented with 8 ng/mL bFGF.

4.2 Change media on human ES cell cultures everyday from Day 3 to Day 7. Monitor colonies to ensure that they are mostly undifferentiated.

4.2 Cells will typically require passaging on Day 6 to 8.

5.0 Immunohistochemical detection of cell surface markers

5.1 Wash the cells twice with 2 mL of PBS.

5.2 Fix the cells with 1 mL of 4% paraformaldehyde for 20 minutes at room temperature.

5.3 Wash the cells twice with 2 mL of PBS for 5 minutes.

5.4 Block the cells with 1 mL of 0.1% BSA, 10% normal goat serum* in PBS at room temperature for 45 minutes.

5.5 During the blocking step, prepare the primary antibody working solution with PBS containing 1% BSA and 10% normal goat serum***** to a final desired concentration.

5.6 After blocking, incubate the cells with 1 mL/well of diluted primary antibody working solution overnight at 2 - 8° C.

5.7 Wash the cells three times with 2 mL of PBS containing 1% BSA for 5 minutes each.

5.8 Dilute the secondary antibody (fluorescence-conjugated) in PBS containing 1% BSA. Incubate the cells with secondary antibody at 1 mL per well for 60 minutes at room temperature in the dark.

5.9 Wash the cells three times with 2 mL of PBS containing 1% BSA for 5 minutes each.

5.10 Cover the cells with 4 mL of PBS and visualize with a fluorescence microscope. Label, date and store plates wrapped in aluminum foil at 4^{o}C.

***** Substitute normal serum from appropriate species depending on the host species of the secondary antibody.

6.0 Embryoid body formation

NOTE: This is a basic protocol for performing spontaneous differentiation studies of human ES cells by removing cells from the substrata and culturing them in suspension as tightly clustered balls of cells referred to as embryoid bodies (EBs). You should optimize your own protocols for this step.

6.1 Human ES cell colonies are dissociated in the same manner as they are when passaging.

6.2 Cells are triturated into small clumps of cells containing ~50 cells/clump and plated on non-adherent 6-well plates in differentiation medium, which contains 20% FBS instead of KnockOut™ Serum Replacement, and no bFGF.

6.3 Cells are allowed to grow in suspension for 4-15 days and then can be re-plated as aggregates or after dissociation, on tissue culture dishes coated with desired substrates or extracellular matrices. Cells can be maintained in DMEM with 20% FBS for additional periods of time during which cells are harvested and analyzed for characteristics of differentiation.

GENERAL GUIDELINES FOR USE

Handle all plates under aseptic conditions.

1. Store Corning® BioCoat™ Matrigel® 6-well plates for ES cell culture at -20°C. **DO NOT THAW AND RE-FREEZE PLATES.** Aseptically remove desired number of plates from packet, re-seal the remaining plates in packet with tape and store at -20°C promptly (within 5 minutes). Re-packaged plates may be stored at -20°C and used for up to two weeks.
2. Thaw plates to room temperature immediately prior to use (~15-30 minutes). Add pre-warmed MEF-CM media and store (1-2 hours maximum) in a 37°C incubator until cells are ready to be plated.
3. Use Corning BioCoat Matrigel 6-well plates for ES cell culture for up to 7-10 days for culturing human ES cells.

STORAGE

Corning BioCoat Matrigel 6-well plates for ES cell culture are stable when stored at -20°C.

CUSTOMER AND TECHNICAL SERVICE

To place an order in the U.S., contact Customer Service at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSCustServ@corning.com.

For technical assistance, contact Technical Support at:
tel: 800.492.1110, fax: 978.442.2476; email: CLSTechServ@corning.com.

Outside the U.S., contact your local distributor or visit www.corning.com/lifesciences to locate your nearest Corning office.

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