GELATIN COATING OF CULTURE PLATES

OBJECTIVE: Human Embryonic Stem Cells (hESCs) are cultured on gelatin-coated plates. Gelatin is a translucent, colorless solid substance extracted from animal collagen. This Standard Operating Procedure (SOP) describes how 0.1% gelatin solution is used to coat 6-well plates for culture of Mouse Embryonic Fibroblasts (MEFs). This SOP can be modified if other culture vessels require gelatin coating.

SCOPE: This procedure applies to all Massachusetts Human Stem Cell Bank laboratory personnel responsible for culture of hESCs.

RESPONSIBILITY: It is the responsibility of the Laboratory Operations Manager and Quality Assurance Officer to ensure all laboratory personnel are properly trained in and follow this SOP.

SAFETY: All laboratory personnel should be in compliance with UMASS Employee Health and Safety regulations when working in the laboratory. Protective equipment, such as a lab coat and disposable gloves must be worn when working in the lab.

ABBREVIATIONS AND DEFINITIONS
hESCs: Human Embryonic Stem Cells
MEF: Mouse Embryonic Fibroblast
SOP: Standard Operating Procedure
UMASS: University of Massachusetts Medical School

REFERENCES
Not applicable

1. MATERIALS REQUIRED

1.1. EQUIPMENT
- Sterile biosafety cabinet (tissue culture hood)
- 37°C incubator
- Pipette-aid

1.2. SUPPLIES
- 6-well tissue culture plates (Costar 3516)
- 5 ml sterile serological pipettes (Costar 4487)
- 10 ml sterile serological pipettes (Costar 4488)
- Sterile Pasteur pipettes (Fisher 13-678-20D)
- Disposable nitrile gloves (World Wide Medical Supplies 71011000-3)

1.3. REAGENTS
1. 0.1% gelatin solution (SOP-RP-001 Preparation of 0.1% Gelatin Solution)
2. PREPARATION

2.1. DOCUMENTATION AND CALCULATION

2. Determine how many culture plates are needed and record on the Log Sheet.
3. Calculate total volume of gelatin solution required according to the total number of wells to be coated. 6-well plates require 2 ml of 0.1% gelatin solution/well.

**Note:** Make sure there is enough 0.1% gelatin solution available. If not, prepare according to SOP-RP-001 Preparation of 0.1% Gelatin Solution.

2.2. PREPARE THE BIOSAFETY CABINET (TISSUE CULTURE HOOD)

1. Place the following items near the hood:
   - 5 ml and 10 ml sterile serological pipettes
   - Absorbent paper towels (or Kimwipes)
   - 70% ethanol spray
   - Appropriate-size disposable gloves
   - Appropriate cell culture plates

3. PROCEDURE

3.1. STERILIZATION PREPARATION BEFORE WORKING IN THE HOOD

1. Wash hands and arms thoroughly (about one minute) with soap.
2. Rinse completely with tap water.
3. Dry hands and arms with paper towel.
4. Put on appropriate-size gloves.
5. Spray 70% ethanol on the gloves and a paper towel until totally wet.
6. Thoroughly clean the working surface in the hood with the ethanol-sprayed paper towel.
7. Spray the surface of everything taken into the hood with ethanol. Dry them with paper towels if needed.

3.2. ALIQUOT 0.1% GELATIN SOLUTION IN THE HOOD

1. Take the bottle of 0.1% gelatin solution from the refrigerator and place it in the hood after cleaning it with 70% ethanol.
2. According to the volume calculated in Section 2.1, do the following:
   - Label an appropriate-size sterile tube or bottle as “GS” for gelatin solution.
   - Transfer an appropriate amount of gelatin solution to the labeled tube or bottle.
3. Place the “GS” tube or bottle in a 37°C water bath or 37°C room for 15-30 minutes.

3.3. COAT CULTURE PLATES USING 0.1% GELATIN SOLUTION

1. Place the plates that are to be coated in the hood.
2. Label the cover of the plate (not over the wells) with:
3. For 6-well plates, add 2 ml of 0.1% gelatin solution to each well.
4. Tilt the plates in several directions so that the liquid covers the entire surface area.
5. Place the plates in a 37°C incubator.

3.4. POST GELATIN COATING
1. Record necessary information on the Gelatin Coating and Inactivated MEF Seeding and Culture Log Sheet.

Note: The plates will be ready for use in 4 hours. They can be used for up to 7 days.