PREPARATION OF 0.1% GELATIN SOLUTION

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 prepared for use of coating cell culture vessels.

SCOPE: This procedure applies to all Massachusetts Human Stem Cell Bank laboratory personnel responsible for preparation of 0.1% gelatin solution.

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
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)
• Mettler balance
• Autoclave

1.2. SUPPLIES
• 500 ml sterile glass bottle (Fisher 02-542C)
• Weigh boat (Fisher brand 08-732-113)
• Disposable nitrile gloves (World Wide Medical Supplies 71011000-3)

1.3. REAGENTS
• Gelatin, Porcine (Sigma G1890)
• Endotoxin-free (MilliQ) water
2. PROCEDURE

2.1. DOCUMENTATION

1. From the binder containing the current Solution/Medium Preparation Log Sheets, take a Gelatin Solution Preparation Log Sheet.
2. Assign a batch number to the solution to be prepared as: GS-yy-mm-dd. For example, for gelatin solution prepared on 10/01/2008, the batch# will be: GS-08-10-01.
3. Record the batch number, date, initials, and the volume to be prepared on the Log Sheet.
4. Take the Log Sheet to the lab to record the manufacturer’s lot number of the gelatin powder that will be used.

2.2. PREPARE 0.1 % GELATIN SOLUTION

Note: Adjust the volume of water and gelatin powder proportionately if a volume other than 500 ml of gelatin is prepared.

1. Place a sterile 500 ml glass bottle that has never been in contact with detergent or soap on the bench.
2. Apply tape to bottle and label as: “0.1% gelatin”, the solution batch#, date and initials.
3. From the chemical shelf, take the bottle of gelatin powder.
4. Record the manufacturer’s lot number of the gelatin powder on the Log Sheet.
5. Weigh 0.5 g of gelatin powder in a weigh boat.
6. Add the 0.5 g of gelatin to the labeled bottle in the hood.
7. Add 500 ml endotoxin-free (e.g., MilliQ) water to the bottle.
8. Swirl the bottle to mix (At this stage, the gelatin is not soluble).
9. Autoclave for 30 minutes (per SOP-SP-003, Sterilization of Solutions) within 2 hours after mixing.
10. Cool the 0.1% gelatin solution to room temperature on bench.
11. Store the 0.1% gelatin solution bottle at 4-8°C in a refrigerator until use. Use this solution within two months of preparation.
12. Return the log sheet to the appropriate binder.
0.1% GELATIN SOLUTION PREPARATION LOG SHEET

Note: Gelatin Solution Batch# is assigned as: GS-yy-mm-dd.  
Expiration date: 2 months from the date it was prepared.

<table>
<thead>
<tr>
<th>Date</th>
<th>Initial</th>
<th>Vol.</th>
<th>Lot #</th>
<th>Expiration Date</th>
<th>Batch #</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>