

## Overlay of Hepatocyte Culture with Basement Membrane Matrix

### Introduction

Though overlay is not necessary for culturing hepatocytes, basement membrane matrix (BMM) overlay will promote a robust hepatocyte culture with 3-dimensional architecture, such as bile canaliculi.

### Purpose

This protocol is to instruct how to apply BMM onto a hepatocyte monolayer.

### Principle of the Procedure

Hepatocytes, freshly plated or cryoplateable, are cultured 4-24 hours to allow for attachment to collagen I coated tissue culture plates prior to BMM overlay.

### Materials\*

Item	Manufacturer	Name
Plated hepatocytes	BioreclamationIVT	
<i>InVitro</i> GRO™ Media	BioreclamationIVT	<i>InVitro</i> GRO CP or HI
Suggested BMM		
Basement Membrane Matrix	Becton Dickenson	Matrigel™
Basement Membrane Matrix	Sigma-Aldrich	ECM Gel
Basement Membrane Matrix	Thermo Fisher	Geltrex®

\*Items listed in this Materials section are for convenience; suitable materials and equipment from other manufacturers may be substituted as appropriate. Contact information for vendors used by BioreclamationIVT are listed in the Notes section at the end of this document.

### Procedure

#### Diluted BMM Medium preparation

1. Obtain protein content of BMM solution.
2. Determine amount of BMM to add to medium to create 0.25 mg/mL.  
e.g. 0.25 mL of BMM (10 mg/mL) into 9.75 mL of medium
3. Thaw Basement Membrane Matrix (BMM) according to manufacturer's instructions.
4. Add appropriate amount of BMM to cold medium (4°C).
5. Mix thoroughly and store on ice.

### **Instructions**

1. Transfer hepatocyte culture plates from incubator to biosafety cabinet.
2. Remove medium from hepatocyte culture plates and add appropriate volume of diluted Matrigel.

6-well: 2.5 mL

12-well: 1 mL

24-well: 0.5 mL

48-well: 0.2 mL

96-well: 0.1 mL

3. Return hepatocyte culture plates to 37°C, 5% CO<sub>2</sub> incubator.
4. Incubate 6-24 hours before next medium change.

Note: For long-term culturing, reapply diluted BMM medium once a week.

### **Cautions**

Treat all products containing human- and monkey-derived materials as potentially infectious, as no known test method can offer assurance that products derived from human or monkey tissues will not transmit infectious agents.

All products are for research use only. Do not use in animals or humans. These products have not been approved for any diagnostic or clinical procedures.