

Assay Methods

Protocol: BD Matrigel Matrix Overlay for Human Hepatocytes

The protocol below describes the BD Matrigel™ matrix overlay procedure for human hepatocytes previously plated in BD BioCoat™ Collagen I-coated plates. Hepatocytes should be plated and cultured for at least 6 hours prior to adding the BD Matrigel matrix overlay.

Note: Follow standard sterility practices for cell culture and applicable Biosafety practices and procedures.

Materials and Equipment:

- BD Matrigel matrix (BD Cat. No. 356237). Prepare single use aliquots by thawing overnight on wet ice, aliquotting and freezing at -20°C.
- Williams' Medium E (WME) without phenol red
- BD™ Hepatocyte Culture Medium Kit (BD Cat. No. 355056)
- 200 mM L-Glutamine
- 50 mg/ml Gentamycin sulfate
- 10 mM Dexamethasone in DMSO or methanol
- BD ITS+ Culture Supplement (BD Cat. No. 354352)
- Biosafety hood
- Cell culture incubator with a 5% CO₂ atmosphere which is appropriate for WME
- Ice bucket
- Positive displacement pipettes and serological pipettes as appropriate for volumes to be transferred

Procedure:

1. Prepare medium for diluting BD Matrigel matrix according to the following ratios.

Component	Volume
WME	500 mL
L-Glutamine	10 mL
Gentamycin Sulfate	0.5 mL
Dexamthasone	0.005 mL
ITS+Supplement	10 mL

2. Medium can be prepared up to 1 month in advance and stored at 4°C.
3. Thaw BD Matrigel matrix on wet ice. This may take up to 8 hours depending on the size of the aliquot.

Note: Thawing overnight may be convenient for your work flow.

4. Prepare a BD Matrigel matrix working solution by diluting chilled BD Matrigel matrix to a final concentration of 0.25 mg/mL with ice cold medium prepared in Step 1.

Note: Prechilled, positive displacement pipettes are recommended due to the viscous nature of BD Matrigel matrix. Mix by inverting the tube three times. Keep this Working Solution on wet ice.

- Aspirate the culture medium from the plated hepatocytes. Add the following volumes of Working Solution to each well with a prechilled positive displacement pipette tip.

Plate Size	Volume per well
6 well	2 mL
12 well	1 mL
24 well	0.5 mL
48 well	0.2 mL
96 well	0.1 mL

- Incubate the overlaid culture at 37°C, in a humidified, 5% CO₂ atmosphere overnight.
- Tilt the plate and carefully aspirate the Working Solution taking care not to disturb the BD Matrigel™ matrix overlay. Replace with the same volume of prewarmed (37°C) BD™ Hepatocyte Culture Medium supplemented with epidermal growth factor (EGF).

Note: With the media aspirated, the hepatocyte monolayer overlaid with BD Matrigel matrix will have a “glossy” appearance relative to a medium only control.

Note: With hepatocytes plated in a BD BioCoat™ Collagen I plate with a BD Matrigel matrix overlay, hepatocytes will have a more rounded morphology relative to hepatocytes plated without overlay.



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