

Corning® Matrigel® Matrix Overlay for Human Hepatocytes

Protocol - Hepatocyte Assay Methods

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Purpose

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

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

Materials and Equipment

- ▶ Corning Matrigel matrix (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
- ▶ Corning Hepatocyte Culture Medium Kit (Cat. No. 355056)
- ▶ 200 mM L-Glutamine
- ▶ 50 mg/mL Gentamycin sulfate
- ▶ 10 mM Dexamethasone in DMSO or methanol
- ▶ Corning ITS+ Culture Supplement (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 Corning 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 Corning 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 Corning Matrigel matrix working solution by diluting chilled Corning 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 Corning 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 Corning® Matrigel® matrix overlay. Replace with the same volume of prewarmed (37°C) Corning Hepatocyte Culture Medium supplemented with epidermal growth factor (EGF).

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

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

Corning acquired the BioCoat™ and Matrigel® brands.

For additional Corning product, technical, or distributor information, please e-mail us at CLSTechServ@corning.com, visit our website www.corning.com/lifesciences or call 800.492.1110. Outside the United States, call 978.442.2200.

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Corning Incorporated Life Sciences

836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476

www.corning.com/lifesciences

Worldwide Support Offices

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LATIN AMERICA

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f (55-11) 3167-0700

Mexico

t (52-81) 8158-8400
f (52-81) 8313-8589

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Corning Incorporated, One Riverfront Plaza, Corning, NY 14831-0001