Corning® Matrigel® Matrix Coating of Transwell® Inserts

Protocol

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Introduction

Corning Matrigel matrix is a solubilized basement membrane preparation extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma. Matrigel matrix is an effective barrier for cell invasion assays. To see sample data using this coating protocol, please refer to Corning application note *Cell Migration and Invasion Quantification Assay with Acetic Acid-dependent Elution of Crystal Violet* (CLS-AN-432).

Materials

- Corning Matrigel matrix (Corning Cat. No. 356234)
- Medium without serum or chemoattractant
- Transwell Permeable Supports (Corning Cat. No. 3384)
- Pipettors
- Sterile tips

Note: Matrigel matrix is a viscous liquid at 4°C but begins to quickly polymerize at higher temperatures (above 10°C). For consistent results, it is essential that the Matrigel matrix is kept cold during use and that any plasticware that will come in contact with the Matrigel matrix is pre-chilled. This includes tips, reservoirs, and the Transwell inserts themselves. For the most accurate pipetting, we recommend using positive displacement pipets or another instrument designed for viscous liquids, such as the Corning Step-R® Repeating Pipettor.

Procedure

Thaw Matrigel matrix by submerging the vial in ice in a 4°C refrigerator, in the back, overnight. Once the Matrigel matrix is thawed, swirl the vial to ensure the material is evenly mixed. Keep the Matrigel matrix cold at all times.

NOTE: The ideal Matrigel matrix coating concentration will be dependent on the invasiveness of your cells, incubation time, and chemoattractant. We recommend optimizing the Matrigel matrix concentration for your cell line and experimental needs. A typical starting range is between 100 µg/cm^2 to 300 µg/cm^2. It is recommended to leave some inserts uncoated for migration controls.

1. Calculate the volume needed to coat desired inserts. Due to its viscosity, there will be some loss of Matrigel matrix, so it is important to make more than the required coating volume.

Table 1. Recommended Coating Volumes

Transwell Permeable Supports	Insert Surface Area (cm^2)	Recommended Coating Volume (mL)
Transwell 96-well HTS	0.143	0.05
Transwell 24-well	0.33	0.1
Transwell 12-well	1.12	0.3
Transwell 6-well	4.67	0.6
Transwell 75 mm insert	44	5

2. Dilute the Matrigel matrix to the desired concentration with medium.

Example Calculations

To coat HTS Transwell 96-well permeable supports at 150 µg/cm^2 from a 9.1 mg/mL vial:

Convert desired coating density (protein/cm^2) to Matrigel matrix concentration (protein/mL):

$$\frac{0.143\ \text{cm}^2 \times 150\ \mu\text{g/cm}^2}{50\ \mu\text{L}} = 0.429\ \mu\text{g/µL}$$

Calculate the total Matrigel matrix needed to make 6 mL of coating solution:

6 mL x 0.429 mg/mL = 2.574 mg of Matrigel matrix

Calculate the volume of stock solution needed:

$$\frac{2.574\ \text{mg}}{9.1\ \text{mg/mL}} = \begin{array}{l}0.28\ \text{mL of 9.1 mg/mL stock solution}\\ 5.72\ \text{mL of serum-free medium}\end{array}$$

3. Add the appropriate amount of diluted Corning® Matrigel® matrix to the Transwell® inserts (Table 1).
4. Incubate plate at 37°C for 1 to 2 hours to polymerize the Matrigel matrix.
5. Seed cells per standard protocol.

For additional information on migration/invasion assays, see the Corning documents listed below:

- Cell Migration, Chemotaxis, and Invasion Assay Protocol (CLS-AN-061)
- Cell Migration, Chemotaxis, and Invasion Assay Using Staining Protocol (CLS-AN-211)
- Considerations when Optimizing your Chemotaxis or Invasion Assay with Corning Transwell Permeable Supports (CLS-AN-188)
- Cell Migration and Invasion Quantification Assay with Acetic Acid-dependent Elution of Crystal Violet (CLS-AN-432)

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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