Laminin Coating Corning® Transwell® Inserts

Protocol

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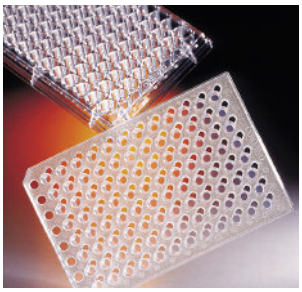

Introduction

There are many procedures that can be used to coat Transwell inserts with fibronectin or other biological coatings. The following is a simple protocol designed to produce a thin coating for cell attachment and cell spreading. Refer to the laminin supplier's protocol for additional coating procedures.

Materials

- Laminin (Sigma-Aldrich® Cat No. L2020)
- Phosphate buffered saline (PBS, without Ca^{++} or Mg^{++}) for rinsing
- Sterile diluting solution (PBS without Ca^{++} or Mg^{++}, or serum-free medium)
- Corning Transwell inserts
- Pipettors and sterile tips

Note: We recommend aliquoting the laminin into multiple stocks so that some can be stored at -20ºC for long term storage, and a working solution can be kept at 4ºC. Avoid repeated freeze-thaw cycles.

Procedure

Note: Different cell lines will require different laminin coating densities in order to obtain the desired results. We recommend optimizing the laminin concentration and coating time for your cell line and experimental needs. We found the best results to be obtained with a range of 10 µg/cm² to 2 µg/cm² using coating times of 30 minutes to 24 hours. In our studies, we found that coating density as well as coating time had a significant impact on cell spreading. Please refer to the protocol *Considerations When Optimizing Coating Protocols for Corning Transwell Permeable Supports* (Sn*APP*Shot, CLS-AN-134), available on the document library at www.corning.com/lifeciences, for more information on this subject.

1. Dilute laminin solution to desired concentration with diluting solution.

   Example of math to coat one 96 well HTS Transwell plate at 10 µg/cm² from a 1 mg/mL working solution:

   Convert desired coating density (laminin/cm²) to laminin concentration (laminin/mL):

   $$\frac{0.143\ \text{cm}^2\ \text{(area of 96 well)} \times 10\ \mu\text{g/cm}^2}{25\ \mu\text{L (volume/96 well)}} = 0.0572\ \mu\text{g/}\mu\text{L} = 0.0572\ \text{mg/mL}$$

   Determine volume of coating solution required to coat one 96 well plate at 25 µL/well:

   25 µL/well x 96 wells = 2.4 mL + 0.6 mL extra = 3 mL of solution

   Calculate total laminin needed to make 3 mL of working solution:

   3 mL x 0.0572 mg/mL = 0.1716 mg of laminin

   Calculate volume of stock solution needed:

   $$\frac{0.1716\ \text{mg}}{1\ \text{mg/mL}} = 0.172\ \text{mL of 1 mg/mL stock solution}$$

   Add 2.828 mL of diluting solution.

2. Add the appropriate amount of diluted laminin solution to the Transwell insert (see Table 1).

Table 1. Recommended Coating and Washing Volumes

Transwell® Insert	Insert Surface Area (cm^2)	Recommended Coating Volume (mL)	Recommended Wash Volume (mL)
96 well HTS	0.143	0.025	0.05
24 well	0.33	0.05	0.1
12 well	1.12	0.25	0.4
6 well	4.67	0.6	1
75 mm insert	44	5	8

3. Incubate inserts at 37ºC for desired coating time (see APPsnote CLS-AN-134 for suggested coating times).
4. Aspirate any remaining laminin solution from the inserts and wash once with PBS or medium (See Table 1). The inserts are now ready for use or can be stored at 4ºC for later use.

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