Collagen Coating Corning® Transwell® Inserts

Protocol

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Corning also offers Transwell-COL inserts that have a transparent, collagen-treated PTFE membrane. The Transwell-COL membrane has an equimolar mixture of types I and III collagen derived from bovine placentas. Unlike traditional coating techniques that result in occluding film layers, this proprietary coating process results in a biologically stable collagen that covers every fibril of the filter matrix, thereby retaining the porosity of the membrane.

Introduction

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

Materials

- Type I Collagen Solution from Rat Tail (Sigma Cat. No. C3867)
- Phosphate buffered saline (PBS)
- Sterile diluting solution (dd$H_2$0, PBS, 70% Ethanol, or 0.02M Acetic Acid)
- Corning Transwell inserts
- Pipettors and sterile tips

Note: Due to the viscosity of most collagen we recommend making a diluted stock solution first and then using this solution to make a working solution at the final desired concentration. If the stock is made at a high concentration such as 2 mg/mL, it can be stored at -20ºC for long term storage or 4ºC for short term storage.

Procedure

Note: Different cell lines will require different collagen coating densities in order to obtain the desired results. We recommend optimizing the collagen concentration and coating time for your cell line and experimental needs. In our studies we found that coating density as well as coating time had a significant impact on cell spreading.

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

   Example: to coat a 96 well HTS Transwell plate at 10 µg/cm² from a 2 mg/mL working solution

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

   $$\frac{0.143\ \text{cm}^2 \times 10\ \mu\text{g/cm}^2}{25\ \mu\text{L}} = 0.0572\ \mu\text{g/uL}$$

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

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

   Calculate volume of stock solution needed:

   $$\frac{0.1716\ \text{mg}}{2\ \text{mg/mL}} = 0.086\ \text{mL of 2 mg/mL stock solution}$$

   Add 2.91 mL of diluting solution for a total of 3 mL.

2. Add the appropriate amount of diluted collagen solution to the Transwell® insert (see table below):

Table 1. Recommended Coating and Washing Volumes

Transwell	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
75mm insert	44	5	8

3. Allow inserts to dry in biological safety cabinet, partially covered.
4. Aspirate any remaining collagen solution from the inserts and rinse once with PBS or cell culture medium (See Table 1). The inserts are now ready for use or can be stored at 4ºC for later use.

For additional product or technical information, please visit **www.corning.com/lifesciences** or call 1.800.492.1110. Outside the United States, please call +1.978.442.2200.

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