

Collagen Coating Transwell® Inserts from Corning

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

- ▶ Collagen I, rat tail
- ▶ Phosphate buffered saline (PBS)
- ▶ Sterile diluting solution (diH₂O, PBS, 70% Ethanol, or 0.02M acetic acid)
- ▶ 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 -20°C for long-term storage or 4°C 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 microplate at 10 µg/cm² from a 2 mg/mL working solution

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

$$\frac{0.143 \text{ cm}^2 \times 10 \text{ µg/cm}^2}{25 \text{ µL}} = 0.0572 \text{ µg/µL}$$

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

$$3 \text{ mL} \times 0.0572 \text{ mg/mL} = 0.1716 \text{ mg of Collagen}$$

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 1).

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
75 mm insert	44	5	8

3. Allow inserts to coat in biological safety cabinet, partially covered for desired amount of time.
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 4°C for later use.

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

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CORNING

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

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

China
t 86 21 3338 4338
f 86 21 3338 4300

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f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6735-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

CSEurope@corning.com

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 020 655 79 28
f 020 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries

t +31 (0) 206 59 60 51
f +31 (0) 206 59 76 73

LATIN AMERICA
grupoLA@corning.com

Brasil
t 55 (11) 3089-7400

Mexico
t (52-81) 8158-8400