

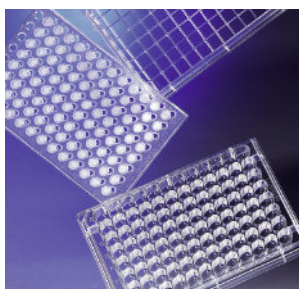
Fibronectin Coating Corning® Transwell® Inserts

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 fibronectin supplier's protocol for additional coating procedures.



Materials

- ▶ Fibronectin from bovine plasma (Sigma-Aldrich® Cat. No. F1141)
- ▶ 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 fibronectin into multiple stocks so that some can be stored at -20°C for long term storage, and a working solution can be kept at 4°C. Avoid repeated freeze-thaw cycles.

Procedure

NOTE: Different cell lines will require different fibronectin coating densities in order to obtain the desired results. We recommend optimizing the fibronectin 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 0.4 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* (SnAPPShot, CLS-AN-134), available in the document library at www.corning.com/lifesciences, for more information on this subject.

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

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

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

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

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

$$25 \text{ } \mu\text{L/well} \times 96 \text{ wells} = 2.4 \text{ mL} + 0.6 \text{ mL extra} = 3 \text{ mL of solution}$$

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

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

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 fibronectin 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
75mm insert	44	5	8

3. Allow inserts to dry in hood, partially covered (see CLS-AN-134 for suggested coating times).
4. Aspirate any remaining fibronectin 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 4°C for later use.

For additional product or technical information, please visit our website at www.corning.com/lifesciences or call 800.492.1110. Customers outside the United States, please call +1.978.442.2200 or contact your local Corning sales office listed below.

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