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**

<table>
<thead>
<tr>
<th>Transwell</th>
<th>Insert Surface Area (cm²)</th>
<th>Recommended Coating Volume (mL)</th>
<th>Recommended Wash Volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well HTS</td>
<td>0.143</td>
<td>0.025</td>
<td>0.05</td>
</tr>
<tr>
<td>24-well</td>
<td>0.33</td>
<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>12-well</td>
<td>1.12</td>
<td>0.25</td>
<td>0.4</td>
</tr>
<tr>
<td>6-well</td>
<td>4.67</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>75 mm insert</td>
<td>44</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

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](http://www.corning.com/lifesciences).

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