

Seeding Cells onto the Underside of Transwell® Permeable Supports from Corning

Guidelines for Use

CORNING

Audrey B. Bergeron and
Ann Rossi, Ph.D.
Corning Incorporated,
Life Sciences
Kennebunk, ME USA

Transwell permeable supports from Corning, which are cell culture inserts containing a permeable membrane, can be used for co-culture of multiple cell types by culturing one cell type in the apical chamber of the Transwell insert and another cell type in the basolateral chamber in a cell culture plate. However, some cell culture models, such as the blood-brain barrier, benefit from the addition of an additional cell type on the underside of the Transwell insert. The following seeding protocol can be applied for 96-, 24-, and 12-well Transwell permeable supports.

Preparing Cell Seeding Suspensions

Cells can be added at the same density per growth area (cells/cm²) that you would typically use for seeding into a Transwell insert. However, the cell density per volume (cells/mL) will need to be adjusted based on the recommended seeding volumes in Table 1. To calculate the seeding concentration, use the calculation below.

$$\text{Seeding concentration (cells/mL)} = (\text{cells/cm}^2 \times \text{growth area}) / \text{seeding volume}$$

Ex: For 96-well format at 1.0×10^5 cells/cm²: $(1.0 \times 10^5 \text{ cells/cm}^2 \times 0.143 \text{ cm}^2) / 0.025 \text{ mL} = 5.72 \times 10^5 \text{ cells/mL}$

Table 1. Transwell insert parameters and recommended coating and seeding volumes

Plate Type	Transwell Insert Diameter	Insert Membrane Growth Area	Recommended Coating/Seeding Volume for Underside of Insert	Recommended Volume for Inside of Transwell Insert	Recommended Volume per Plate Well
96-well	4.26 mm	0.143 cm ²	25 µL	75 µL	235 µL
24-well	6.5 mm	0.33 cm ²	50 µL	100 µL	600 µL
12-well	12 mm	1.12 cm ²	200 µL	500 µL	1500 µL

Cell Seeding onto the Underside of Transwell Permeable Supports

1. After cell suspensions are prepared, turn the Transwell plate system upside-down so that it is resting on its lid. Lift off the reservoir or multiwell plate from the inserts, taking care to not touch the inserts or the inside of the plate. If you are using the HTS reservoir plate, leave the gridded insert with the Transwell inserts.
2. Using a single- or multi-channel pipet, add 25 µL of cell suspension to the underside of each Transwell membrane (Figure 1). If using 24- or 12-well Transwell systems, adjust the seeding volume using the recommended volume in Table 1.

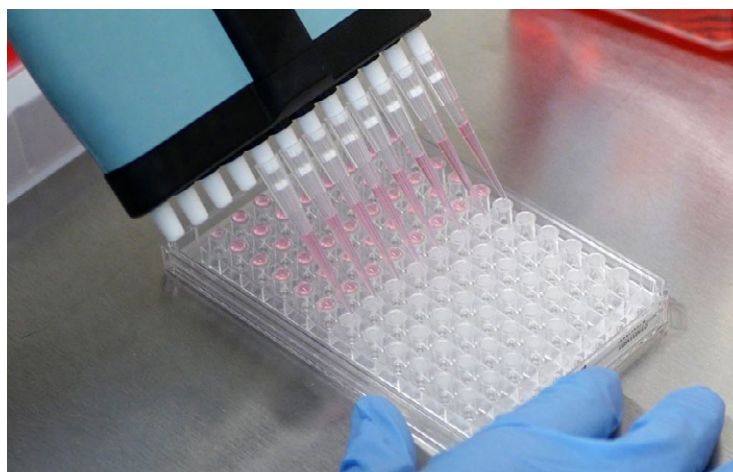


Figure 1. Cell seeding onto the underside of Transwell inserts. Droplets of cell suspension or coating solutions can be applied to the underside of Transwell inserts using a single- or multi-channel pipet.

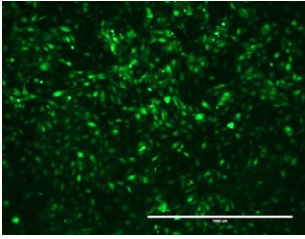


Figure 2. Imaging of fluorescent cells seeded on the underside of Transwell inserts. GFP-expressing human umbilical vein endothelial cells (HUVEC-GFP) were seeded at 70K cells/cm² on 1 μm PET membrane in EGM™-Plus Endothelial Cell Grow Medium (Lonza Cat. No. CC-5035) and incubated overnight in a humidified 37°C, 5% CO₂ incubator. Representative image was taken with an EVOS® FL microscope with 4X objective. Scale bar = 100 μm.

3. Leave the Transwell® inserts upside-down in the biosafety cabinet for 30 minutes to 1 hour to allow time for the cells to settle and attach to the membrane. You can cover the inserts with a sterile empty tip box lid to help maintain an aseptic environment during incubation.
4. After incubation, carefully replace the reservoir or multiwell plate over the inserts. Then, turn over the Transwell system so that it is in the standard cell culture position.

NOTE: Culture media may drop from the membranes into the reservoir/multiwell plate. If you are concerned about cells attaching to the bottom plate, use either a non-treated plate or switch to a clean plate after the cell attachment phase.

5. Add culture media or another cell type to the apical chamber inside the Transwell inserts using the recommended volume in Table 1.
6. Add culture media to the basolateral chamber in the plate using the recommended volume in Table 1. If you are using a reservoir with the HTS Transwell-96, add 25 mL culture media to the reservoir.
7. Incubate under standard cell culture conditions (i.e., at 37°C, 5% CO₂ in a humidified incubator) until desired cell confluence is achieved (Figure 2).

NOTE: It may be difficult to view cells on the underside of Transwell inserts using bright-field or phase contrast microscopy due to difficulty focusing. For visualization during cell seeding optimization, it is recommended to use fluorescently labeled, fluorescently stained, or crystal violet stained cells.

Coating the Underside of Transwell Permeable Supports

Some cell types may require the addition of a coating to adhere to the Transwell permeable membranes. The same concentration of coating per cm² that is typically used in standard culture can be added to the underside of the Transwell inserts in a small droplet. However, the coating concentration (μg/mL) will need to be adjusted based on the recommended coating volumes in Table 1. To calculate the coating concentration, use the calculation below.

Coating concentration (μg/mL) = (X μg/cm² x growth area)/coating volume

Ex: For 96-well format at 10 μg/cm²: (10 μg/cm² x 0.143 cm²)/0.025 mL = 57.2 μg/mL

The same protocol that is used for cell seeding onto the underside of Transwell inserts can also be used for coating prior to cell seeding.

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

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.

For additional product or technical information, visit www.corning.com/lifesciences or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

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

India
t 91 124 4604000
f 91 124 4604099

Japan

t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
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

CEurope@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