

# Transwell® Permeable Supports

Including Snapwell™ and Netwell™ Inserts

Instructions for Use

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## INTRODUCTION

Transwell® Permeable Supports are convenient and easy-to-use devices for studies of both anchorage-dependent and anchorage-independent cells. These inserts provide independent access to both sides of a monolayer, thus giving researchers a versatile tool to study transport and other metabolic activities *in vitro*. Transwell units are available in a range of diameters, membrane types and pore sizes to satisfy a variety of research requirements.

## TRANSWELL INSERT PRODUCT DESCRIPTIONS



### Standard Transwell Inserts

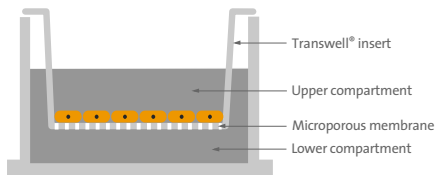
Standard Transwell inserts are available in four membrane diameters: 6.5 mm (24 well plate), 12 mm (12 well plate), 24 mm (6 well plate) and 75 mm (100 mm dish) formats. See Table 1 for cell growth areas.

**Table 1. Transwell Permeable Support Growth Areas\***

Transwell Insert Diameter	Multiple Well Plate or Dish Style	Insert Membrane Growth Area
6.5 mm	24 well	0.33 cm <sup>2</sup>
12 mm	12 well	1.12 cm <sup>2</sup>
24 mm	6 well	4.67 cm <sup>2</sup>
75 mm	100 mm dish	44 cm <sup>2</sup>

\* Values are reported as nominal and may vary slightly due to inherent variability of our manufacturing process. To insure success, we recommend that end users validate their methods independent from our reported values.

Several membrane types and a large selection of pore sizes are available with each of these units. A patented\* self-centering design prevents medium from



wicking between the sides of the insert and the well wall. The hanging design keeps the Transwell® membrane about a millimeter off the bottom of the well. This prevents co-cultured cell monolayers in the bottom of the well from being scratched or disturbed when the insert is moved. Windows or openings in the sides of the Transwell insert allow access to the lower compartment.

## Membrane Types

Transwell permeable supports are available in three membrane materials: polycarbonate, polyester (PET), and collagen-coated polytetrafluoroethylene (PTFE). See Table 2 for additional information on these membrane characteristics.

**Table 2. Characteristics of Transwell Membranes**

Characteristics	Polyester (PET)	Polycarbonate	PTFE/ Collagen-coated
Optical properties	Clear	Translucent	Clear when wet
Cell visibility	Good	Poor	Cell outlines
Tissue culture treated	Yes	Yes	No
Nominal Membrane thickness <sup>‡</sup>	10 µm	10 µm	30 µm
Matrix/ECM coatable	Yes	Yes	Yes
Collagen treated	No	No	Yes
Available Pore Sizes (µm)	0.4, 3.0, 0.8	0.4, 3.0, 5.0, 8.0, 12.0	0.4, 3.0

<sup>‡</sup>Membrane manufacturing processes are inherently variable; thus, actual values may vary up to 60% of nominal depending on membrane type and measurement techniques.

\*Patent number 5,026,649.

Polycarbonate Transwell® inserts feature thin, translucent membranes available in six pore sizes ranging from 0.1 µm to 12.0 µm. Most are treated for optimal cell attachment. They are supplied sterile and come preloaded in multiple well plates or dishes. The polycarbonate membrane is compatible with most organic fixatives and stains.

Polyester (PET) Transwell-Clear inserts have microscopically transparent polyester membranes that are tissue culture treated for optimal cell attachment and growth. Transwell-Clear inserts provide better cell visibility under phase contrast microscopy and allow assessment of cell viability and monolayer formation.

Transwell-COL inserts have transparent (when wet), collagen-treated PTFE membranes that promote cell attachment and spreading and allow cells to be visualized during culture. The Transwell-COL membranes are coated with an equimolar mixture of types I and III collagen derived from bovine placentas. Unlike traditional coating techniques that result in occluding film layers, Corning's proprietary coating process results in a biologically stable collagen that covers every fibril of the filter matrix, thereby retaining the porosity of the membrane.

## Pore Sizes

Selecting the correct pore size for experiments is also very important. The smaller pore size Transwell membranes (0.4 or 3.0 µm) are primarily used in drug transport studies. Cell invasion, chemotaxis and motility studies are usually done in Transwell membranes with 3.0 µm or larger pores. The ability of cells to migrate through pores of a membrane is dependent on the cell line used and the culture conditions, as well as the pore size. Cell migration will not occur with pores smaller than 3.0 µm. For critical experiments, we suggest testing with appropriate controls and a range of pore sizes to determine which size works best with your cell cultures and your specific application. As an alternative, follow recommendations from the scientific literature. For additional application and use information, please refer to the Transwell

inserts bibliography on the Technical Information section of the Corning Life Sciences web site ([www.corning.com/lifesciences](http://www.corning.com/lifesciences)) that lists over 800 literature references using Transwell permeable supports.

### Pore Density

Of the three types of Transwell® membranes, only the PTFE membrane does not have a defined pore density because it is a tortuous path membrane. The two membranes with nominally defined pore densities are polycarbonate and polyester. The polyester Transwell membranes do not have as high a pore density as the polycarbonate Transwell membranes but they have better optical clarity as a result. The nominal pore densities for polycarbonate and polyester membranes are given in Table 3.

**Table 3. Nominal Pore Densities for Transwell Polyester and Polycarbonate Membranes**

Pore Size	Nominal Pore Density	
	Polycarbonate Membrane	Polyester Membrane
0.4 µm	1 x 10 <sup>8</sup> pores/cm <sup>2</sup>	4 x 10 <sup>6</sup> pores/cm <sup>2</sup>
1.0 µm	–	1.6 x 10 <sup>6</sup> pores/cm <sup>2</sup>
3.0 µm	2 x 10 <sup>6</sup> pores/cm <sup>2</sup>	2 x 10 <sup>6</sup> pores/cm <sup>2</sup>
5.0 µm	4 x 10 <sup>5</sup> pores/cm <sup>2</sup>	–
8.0 µm	1 x 10 <sup>5</sup> pores/cm <sup>2</sup>	1 x 10 <sup>5</sup> pores/cm <sup>2</sup>
12.0 µm	1 x 10 <sup>5</sup> pores/cm <sup>2</sup>	–

### Chemical Compatibility

All of the Transwell membranes are compatible with histological fixatives including methanol and formaldehyde. The polyester Transwell membranes have the best overall chemical resistance. These membranes (but not the polystyrene housings) are compatible with many alcohols, amines, esters, ethers, ketones, oils and some solvents including many halogenated hydrocarbons and DMSO, but are not recommended for use with strong acids and bases.

## USING TRANSWELL® PERMEABLE SUPPORTS

### Directions for Use

1. Transwell inserts are used by first adding medium to the multiple well plate well, followed by adding the Transwell inserts, and lastly adding the medium and cells to the inside compartment. Recommended medium volumes are shown in Table 4.

**Table 4. Recommended Transwell Permeable Support Medium Volumes**

Transwell Insert Diameter	Insert Membrane Growth Area	Multiple Well Plate or Dish Type	Volume Added per Plate Well	Volume Added to Inside of Transwell Insert
4.26 mm	0.143 cm <sup>2</sup>	96 well	0.235 mL	0.075 mL
6.5 mm	0.33 cm <sup>2</sup>	24 well	0.6 mL	0.1 mL
12 mm	1.12 cm <sup>2</sup>	12 well	1.5 mL	0.5 mL
24 mm	4.67 cm <sup>2</sup>	6 well	2.6 mL	1.5 mL
75 mm	44 cm <sup>2</sup>	100 mm dish	13 mL	9.0 mL

2. An initial equilibrium period may be used to improve cell attachment by adding medium to the multiple well plate well and then to the Transwell insert. The plate should then be incubated for at least one hour or even overnight at the same temperature that will be used to grow the cells. The cells are then added in fresh medium to the Transwell insert and returned to the incubator.
3. The medium level should be checked periodically and fresh medium added as required.
4. Transwell inserts have three openings for standard pipette tips to allow samples to be added or removed from the lower compartment.
5. Cell monolayers may be fixed and stained while in the Transwell insert using standard cytological techniques. Avoid using solvents that dissolve polystyrene or polycarbonate or polyester membrane materials. Processing steps may be carried out by

sequentially moving the Transwell insert through a series of multiple well plate wells containing the appropriate reagents. Protocols for fixing and staining Transwell® inserts are available on the Corning Life Sciences web site ([www.corning.com/lifesciences](http://www.corning.com/lifesciences)).

6. If it is necessary to remove cells from Transwell membranes, we recommend rinsing both the Transwell insert and the plate well. Then the dissociating solution should be added to both the well and the Transwell insert and incubated until the cells begin to come off. A protocol, *Trypsinization Procedure for Corning® Transwell® Inserts*, for removing cells from Transwell inserts is available in the technical section of the Corning Life Sciences web site.
7. The polycarbonate or polyester membrane with the fixed and stained cells attached may be removed from the Transwell insert by carefully cutting around the membrane edges with a scalpel.
8. The collagen-coated PTFE membrane is fragile and requires careful handling during removal. A wetted cellulosic membrane filter should be placed in direct contact with the underside of the Transwell insert membrane before it is cut out with a scalpel. The wetted, more rigid, cellulosic filter will serve as a support for the collagen-coated membrane.

### Helpful Hints

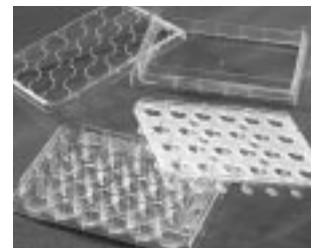
1. Cell morphology and cell densities on permeable supports are influenced by filter pore size.
2. Larger pore sizes may permit some cell types to migrate through the pores on the permeable support.
3. Cells grown on permeable supports are often sensitive to initial seeding density for good cell attachment. On first use, try bracketing a range of seeding densities for optimum growth.
4. Cell attachment and spreading may be improved by preincubating permeable supports in medium prior to seeding.

5. Cells requiring extracellular matrix coatings on plastic substrates will also require them on permeable supports.
6. The Transwell®-Clear insert contains a transparent tissue culture treated polyester membrane that allows easy viewing of cells using phase contrast microscopy.
7. The Transwell-COL insert contains a PTFE membrane that has been treated with an equimolar mixture of types I and III bovine placental collagens. This results in a biologically stabilized collagen matrix covering the fibrils of the filter membrane. These Transwell inserts are excellent for the growth of cells requiring a biological coating.

## ADDITIONAL PERMEABLE SUPPORT PRODUCTS

### HTS Transwell-24 System

The HTS Transwell-24 System is an array of 24 individual Transwell inserts (6.5 mm diameter membranes) connected by a rigid, robotics-friendly holder that enables all 24 Transwell inserts to be handled as a single unit. This makes the HTS Transwell-24 System an ideal tool for running automated, high throughput drug transport (Caco-2 cells) or cell toxicity studies. The tissue culture treated polycarbonate membrane is available in either 0.4  $\mu\text{m}$  or 3.0  $\mu\text{m}$  pore sizes. The tissue culture treated polyester membrane is available in 0.4  $\mu\text{m}$  pore size. Both membrane types provide an excellent substrate for cell attachment, growth, and differentiation. An open culture reservoir plate is used to reduce liquid handling during cell feeding (medium can be exchanged all at once). Once the cell layers are confluent, the HTS Transwell-24 insert is transferred to a 24 well plate for running experiments.



## HTS Transwell®-96 Permeable Support Systems and Plates

The HTS Transwell-96 product is an array of 96 Transwell inserts integrated into a single robotics-friendly unit. Plates are available with either polyester (PET) membrane or polycarbonate (PC) membrane.

All standard units are tissue culture treated (membrane only) and sterile. The HTS Transwell-96 Permeable Supports have a well bottom elevation of 1.3 mm.

The HTS Transwell-96 Systems (0.4 µm PC and 1.0 µm PET) are designed for drug transport, excretion, and other ADMET assays. The systems are packaged with the 96 well insert plate in a reservoir plate and includes the 96 well receiver plate with lid. The HTS Transwell-96 Well Plates (3.0 µm PC, 5.0 µm PC, 8.0 µm PET) are ideal for chemotaxis, migration, and invasion assays. These are packaged with the 96 well insert plate in the 96 well receiver plate with lid.

## Snapwell™ Inserts

The Snapwell insert is a modified Transwell culture insert that contains a 12 mm diameter tissue culture treated polycarbonate or clear polyester membrane supported by a detachable ring. These inserts are primarily used for transport and electrophysiological studies. Once cells are grown to confluence, this ring-supported membrane can be placed into either vertical or horizontal diffusion or Ussing chambers. Chambers are available from Harvard Apparatus, [www.harvardapparatus.com](http://www.harvardapparatus.com).



\*Patent numbers 5,026,649 and 5,139,951.

## Netwell™ Inserts

Netwell inserts are polystyrene inserts fitted with polyester mesh bottoms for use with 6 and 12 well plates.

### Typical Applications

- ▶ For 3-D tissue culture, the sterile Netwell insert provides efficient media permeation and support to cultures grown in gels
- ▶ Sterile Netwell inserts provide easy processing of tissue explants and microcarrier-grown cells
- ▶ Modular design makes the Netwell insert handy for immunocytochemical staining on multiple sections

## TECHNICAL ASSISTANCE

For additional product or technical information, please visit [www.corning.com/lifesciences](http://www.corning.com/lifesciences) or call 1.800.492.1110. The web site contains protocols and a bibliography with over 800 references for using Transwell® permeable supports. Customers outside the United States, please call +1.978.635.2200.

## ORDERING INFORMATION

### Polycarbonate Membrane Transwell® Inserts

Cat. No.	Membrane Diameter (mm)	Growth Surface Area (cm <sup>2</sup> )	Membrane Pore Size (µm)	Tissue Culture Treated	Inner Packaging	Inserts/Case
3413	6.5	0.33	0.4	Yes	12/plate*	48
3415	6.5	0.33	3.0	Yes	12/plate*	48
3421	6.5	0.33	5.0	Yes	12/plate*	48
3422	6.5	0.33	8.0	Yes	12/plate*	48
3401	12	1.12	0.4	Yes	12/plate	48
3402	12	1.12	3.0	Yes	12/plate	48
3403	12	1.12	12.0	Yes	12/plate	48
3412	24	4.67	0.4	Yes	6/plate	24
3414	24	4.67	3.0	Yes	6/plate	24
3428	24	4.67	8.0	Yes	6/plate	24
3419	75	44	0.4	Yes	1/dish	12
3420	75	44	3.0	Yes	1/dish	12

\*Packaged 12 inserts per 24 well plate

### Polyester (PET) Membrane Transwell-Clear Inserts

Cat. No.	Membrane Diameter (mm)	Growth Surface Area (cm <sup>2</sup> )	Membrane Pore Size (µm)	Inner Packaging	Inserts/Case
3470	6.5	0.33	0.4	12/plate*	48
3472	6.5	0.33	3.0	12/plate*	48
3460	12	1.12	0.4	12/plate	48
3462	12	1.12	3.0	12/plate	48
3450	24	4.67	0.4	6/plate	24
3452	24	4.67	3.0	6/plate	24

\*Packaged 12 inserts per 24 well plate

### Collagen-Coated Transwell®-COL Inserts

Cat. No.	Membrane Diameter (mm)	Growth Surface Area (cm <sup>2</sup> )	Membrane Pore Size (µm)	Inner Packaging	Multiple Well Plate	Inserts/Case
3495	6.5	0.33	0.4	Individual	24 well	24
3496	6.5	0.33	3.0	Individual	24 well	24
3493	12	1.12	0.4	Individual	12 well	24
3494	12	1.12	3.0	Individual	12 well	24
3491	24	4.67	0.4	Individual	6 well	24
3492	24	4.67	3.0	Individual	6 well	24

### HTS Transwell-24 Systems

Cat. No.	Description	Pore Size (µm)	Qty/Pk	Qty/Cs
3378	HTS Transwell-24, PET membrane, Bulk	0.4	12	12
3379	HTS Transwell-24, PET membrane, Individual	0.4	1	2
3395	HTS Transwell Open Culture Reservoir	N/A	12	48
3396	HTS Transwell-24, Individual	0.4	1	2
3397	HTS Transwell-24, Bulk	0.4	12	12
3398	HTS Transwell-24, Individual	3.0	1	2
3399	HTS Transwell-24, Bulk	3.0	12	12

## ORDERING INFORMATION (CONTINUED)

### HTS Transwell®-96 Well Permeable Supports

Cat. No.	Description	Pore Size (µm)	Membrane	Qty/Pk	Qty/Cs
3380	HTS Transwell-96 System	1.0	PET	1	1
3392	HTS Transwell-96 System	1.0	PET	1	5
3381	HTS Transwell-96 System	0.4	PC	1	1
3391	HTS Transwell-96 System	0.4	PC	1	5
3385	HTS Transwell-96 Well Plate	3.0	PC	1	2
3386	HTS Transwell-96 Well Plate	3.0	PC	4	8
3387	HTS Transwell-96 Well Plate	5.0	PC	4	8
3388	HTS Transwell-96 Well Plate	5.0	PC	1	2
3374	HTS Transwell-96 Well Plate	8.0	PET	1	2
3384	HTS Transwell-96 Well Plate	8.0	PET	4	8
3382	HTS Transwell-96 Receiver Plate with lid	N/A	N/A	10	10
3383	HTS Transwell-96 Feeder Reservoir with media stabilizer and lid	N/A	N/A	10	10
3583	HTS Transwell-96 black receiver plate with lid, TCT	N/A	N/A	10	10

### Snapwell™ Inserts

Cat. No.	Membrane Pore Size (µm)	Membrane Material	Inner Packaging	Inserts/Case
3407	0.4	Polycarbonate	6/plate	24
3801	0.4	Clear Polyester	6/plate	24
3802	3.0	Polycarbonate	6/plate	24

### Netwell™ Inserts

Cat. No.	Membrane diameter (mm)	Membrane Mesh Size (µm)	Sterile	Inner Packaging	Inserts/Case
3477	15	74	Yes	12/plate	48
3478	15	500	Yes	12/plate	48
3479	24	74	Yes	6/plate	48
3480	24	500	Yes	6/plate	48

### Netwell™ Accessories

Cat. No.	Description	Inserts/Case
3517	Netwell reagent tray, black	200
3519	Netwell reagent tray, white	200
3520	Netwell carrier kit*, 15 mm	8
3521	Netwell carrier kit*, 24 mm	8

\*Kits contain eight carriers and eight handles.

### 6, 12, and 24 Well Cell Culture Plates

Cat. No.	Number of Wells	Well Diameter (mm)	Growth Area (cm <sup>2</sup> )	Qty/Pk	Qty/Cs
3506	6	34.8	9.5	5	100
3516	6	34.8	9.5	1	50
3512	12	22.1	3.8	5	100
3513	12	22.1	3.8	1	50
3524	24	15.6	1.9	1	100
3526	24	15.6	1.9	1	50
3527	24	15.6	1.9	5	100



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