APPLICATIONS FOR FALCON® CELL CULTURE INSERTS

MEMBRANE TYPE	MEMBRANE CHARACTERISTICS	TYPICAL APPLICATIONS
0.4 micron PET 3.0 micron PET	■ Strong ■ Transparent ■ Will not curl when removed from housing	■ Immunofluorescence ■ Direct visualization of cells by light microscopy ■ Electron microscopy and confocal microscopy ■ Coculture
0.4 micron high pore density (HD) PET	■ Translucent ■ Porosity equal to competitive 0.4 µm track-etched polycarbonate membranes ■ High rates of basolateral diffusion	■ Vectorial transport ■ Binding, secretion ■ Coculture ■ *In vitro* toxicology
1.0 micron PET	■ Transparent ■ Porosity and permeability equivalent to 0.4 µm HD membranes ■ Good basolateral feeding, diffusion	■ Vectorial transport ■ Tissue modeling and differentiation ■ Direct visualization of cells by light microscopy ■ Binding, secretion ■ Coculture ■ *In vitro* toxicology
3.0 micron high pore density (HD) PET	■ High porosity, high permeability ■ Rapid diffusion of large molecules, such as lipoproteins, virus and bacteria ■ High rates of basolateral diffusion	■ Vectorial transport ■ Binding, secretion
8.0 micron PET	■ High porosity, high permeability ■ Large pores allow passage of mammalian cells	■ Chemotaxis ■ Invasion, metastasis ■ Transendothelial migration

TECHNICAL BULLETINS AVAILABLE FROM CORNING

Number	Author	Title
401	Kurt Amsler Vedrana Cijvojc John H. Durham	Maintenance and Functional Properties of Primary Turtle Bladder Epithelial Cells Cultured on Falcon Cell Culture Inserts (CLS-DL-CC-060)
402	Elizabeth J. Roemer Sanford R. Simon	Falcon Cell Culture Inserts as a Supportive Substrate for an *In Vitro* Extracellular Matrix System (CLS-DL-CC-061)
404	Kurt Amsler Harry Gray	Gamma-Glutamyl Transpeptidase Assay: An Example of a Protocol for Determining the Sidedness of Asymmetrical Expression of a Membrane Protein, Enzyme, or Transport Activity in an Epithelial or Other Cell Type
405	Harry Gray Oresta Fedun	Preparation of Falcon Cell Culture Inserts for Scanning Electron Microscopy (CLS-DL-CC-062)
406	Mary Gray Fred Morris	Preparation of Falcon Cell Culture Inserts for Transmission Electron Microscopy (CLS-DL-CC-063)
407	Elizabeth Roemer	An *In Vitro* Assay for Study of Neutrophil Migration Through Interstitial Matrix Using Falcon Cell Culture Inserts (CLS-DL-CC-064)
408	Barbara J. Johnson	Introduction of Lymphoproliferation by Antigen-primed Macrophage across Falcon Cell Culture Inserts (CLS-DL-CC-065

To obtain any of these technical bulletins or for additional technical information please call 1-978-442-2200.

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GUIDELINES FOR USING Falcon® Cell Culture Inserts

INTRODUCTION

CORNING offers a broad line of cell culture inserts incorporating polyethylene terephthalate (PET) track-etched membranes. Perfectly transparent, low pore density PET membranes provide a durable substrate for light microscopy, electron microscopy and immunofluorescence. These exceptionally strong membranes can be removed for staining, fixing or other procedures. Once removed, the membrane will not curl and remains easy to handle.

High pore density (HD) translucent PET membranes are more highly permeable substrates. They allow increased rates of basolateral diffusion of nutrients and molecules of interest for transport, secretion or binding studies.

Track-etched membranes have symmetrical, cylindrical pores. Both sides of the membrane are tissue culture-treated (TC) and are suitable for cell growth.

Refer to the complete listing of Falcon Cell Culture Inserts below.

For information about our Corning BioCoat™ extracellular matrix and matrix component inserts, please call 1-978-442-2200.

TABLE 1
Falcon Cell Culture Inserts

CATALOG NO.	MEMBRANE MATERIAL	PORE SIZE (MICRON)	PORE DENSITY (PORES/SQ. CM)	OPTICAL QUALITY	TC PLATE (NO. OF WELLS)
353090	PET	0.4	$2.0 \pm 0.2 \times 10^6$ / cm^2	TRANSPARENT	6
353180	PET	0.4	$2.0 \pm 0.2 \times 10^6$ / cm^2	TRANSPARENT	12
353095	PET	0.4	$2.0 \pm 0.2 \times 10^6$ / cm^2	TRANSPARENT	24
353102	PET	1.0	$1.6 \pm 0.6 \times 10^6$ / cm^2	TRANSPARENT	6
353103	PET	1.0	$1.6 \pm 0.6 \times 10^6$ / cm^2	TRANSPARENT	12
353104	PET	1.0	$1.6 \pm 0.6 \times 10^6$ / cm^2	TRANSPARENT	24
353091	PET	3.0	$8 \pm 2 \times 10^5$ / cm^2	TRANSPARENT	6
353181	PET	3.0	$8 \pm 2 \times 10^5$ / cm^2	TRANSPARENT	12
353096	PET	3.0	$8 \pm 2 \times 10^5$ / cm^2	TRANSPARENT	24
353093	PET	8.0	$6 \pm 2 \times 10^4$ / cm^2	TRANSLUCENT	6
353182	PET	8.0	$6 \pm 2 \times 10^4$ / cm^2	TRANSLUCENT	12
353097	PET	8.0	$6 \pm 2 \times 10^4$ / cm^2	TRANSLUCENT	24
353493	PET	0.4HD	$100 \pm 10 \times 10^6$ / cm^2	TRANSLUCENT	6
353494	PET	0.4HD	$100 \pm 10 \times 10^6$ / cm^2	TRANSLUCENT	12
353495	PET	0.4HD	$100 \pm 10 \times 10^6$ / cm^2	TRANSLUCENT	24
353092	PET	3.0HD	$2.0 \pm 0.2 \times 10^5$ / cm^2	TRANSLUCENT	6
353292	PET	3.0HD	$2.0 \pm 0.2 \times 10^5$ / cm^2	TRANSLUCENT	12
353492	PET	3.0HD	$2.0 \pm 0.2 \times 10^5$ / cm^2	TRANSLUCENT	24

NOTES: *All products are sterilized by gamma irradiation and are intended for single use only.*
HD signifies a high pore density membrane for maximum permeability.
PET is tissue culture treated polyethylene terephthalate.

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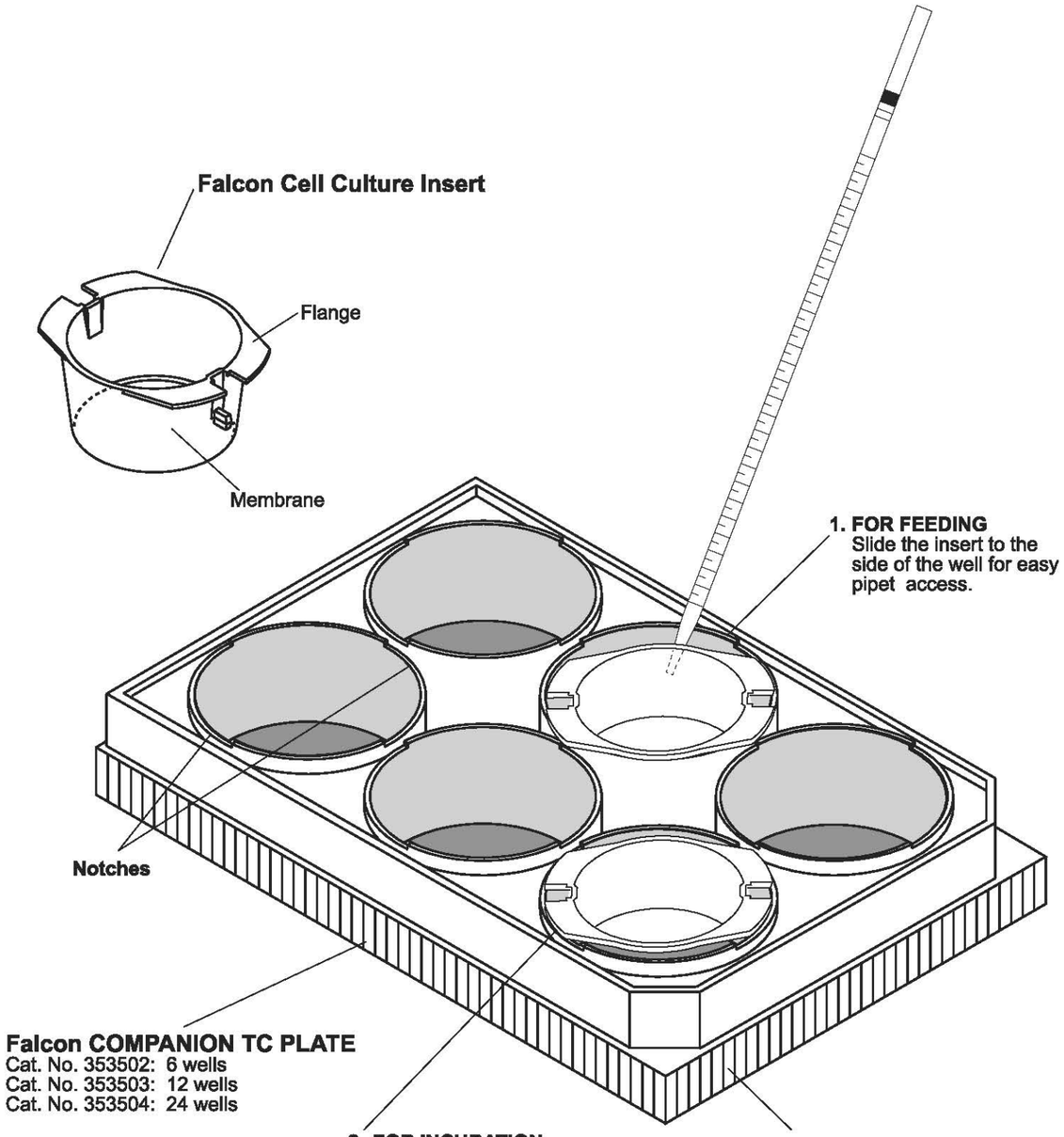
DIAGRAM A
Falcon® Cell Culture Insert
Falcon Insert used with a Falcon Companion Tissue Culture Plate



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DIRECTIONS FOR USING FALCON® CELL CULTURE INSERTS

Handle inserts under aseptic conditions.

1. Add prewarmed culture medium to each well of a multiwell tissue culture plate. Refer to Table 2 for recommended working volumes.

2. Aseptically, open the package, remove an insert with sterile forceps, and gently place it into the well. Avoid trapping air under the insert by tilting the insert while lowering it into the well.

   If you use Falcon Cell Culture Insert Companion Plates, position inserts with the flanges resting in the notches on the top edge of each well. This will position inserts diagonally as shown in Diagram A.

3. **Seeding**

   It is recommended to store cell culture medium in incubator for 20 min. before seeding for pH equilibrium.

   Add cells and media to the insert, referring to Table 2 for recommended working volumes. To determine the optimal seeding density for your cell type on a porous growth surface, we recommend using a range of seeding densities (cells/sq. cm) that brackets the seeding density used on nonporous surfaces (flasks, dishes and plates). For example: if you currently seed at 10^5 cells per sq. cm, seed at 0.5 x 10^5, 10^5 and 5x10^5 to determine the optimal initial seeding density. Refer to Table 2 for surface areas of inserts and wells.

4. **Initial Attachment and Cell Culture**

   Culture your cells under routine conditions. For some cells, initial attachment and lag phase may vary with insert material, pore size and pore density. After initial attachment, growth rates (doubling times) will generally be equivalent with equivalent times to confluency.

5. **Microscopy**

   If you use transparent, low pore density membranes you can observe your live cultures using routine phase contrast or bright field microscopy. Large pore size and high pore density membranes may appear "speckled" due to shadows being cast by the pores.

6. **Feeding**

   Use a standard 1 mL or pasteur pipet to remove media from above and below the membrane.

   If you use Falcon Cell Culture Insert Companion Plates, first slide inserts to one side as shown in Diagram A using a sterile pipet or forceps. This will provide better pipet access for aspirating and replacing media.

   Replace media with appropriate size pipet.

   Reposition inserts in the notches for incubation.

   Use of Falcon Cell Culture Insert Companion Plates may allow you to use a larger diameter (volume) pipet when dispensing media.

7. **Retrieving Cells**

   To remove cells, follow your standard trypsinization or scraping procedure. Smaller diameter inserts can be scraped with a small rubber policeman or the blunt end of a pasteur pipet.

   Note: When using larger pore size membranes, some liquid may drip through the membrane. This should be considered during trypsinization.

8. **Fixing and Staining**

   Cells can be fixed using standard techniques. Inserts can be processed intact, by passing them through a series of fixation solutions. The membrane can easily be removed from the housing by cutting with a razor blade or scalpel to prepare sections for embedding, sectioning or staining. Inserts are stable under most processing conditions, and are recommended for TEM and SEM as described in Falcon Technical Bulletin Nos. 405 and 406.

9. **Extracellular Matrix**

   The use of extracelluar matrix proteins with porous supports provides a highly relevant in vitro model. A full line of matrix proteins, Corning® BioCoat™ precoated growth vessels and Corning BioCoat precoated cell culture inserts is available from **CORNING**. For information about these products, please call 1-978-442-2200.

TABLE 2
Falcon Cell Culture Inserts and Companion Plates
Physical Specifications

	6 Well	12 Well	24 Well
EFFECTIVE DIAMETER OF MEMBRANE (mm)	23.1	10.5	6.4
EFFECTIVE GROWTH AREA OF MEMBRANE (cm^2)	4.2	0.9	0.3
INSERT HEIGHT (mm)	17.2	17.2	17.5
DISTANCE FROM MEMBRANE TO THE BOTTOM OF WELL (mm)	0.9	0.9	0.8
SUGGESTED MEDIA IN INSERT (mL)	1.5 - 2.5	0.4 - 1.0	0.2 - 0.35
SUGGESTED MEDIA IN WELL (mL)	2.7 - 3.2	1.4 - 2.3	0.7 - 0.9
INSERT CASE QUANTITY	48	48	48
Falcon COMPANION TISSUE CULTURE PLATE CATALOG NUMBER	353502	353503	353504
GROWTH AREA IN TC PLATE WELL (cm^2)	9.6	3.8	2.0
COMPANION PLATE CASE QUANTITY	50	50	50
COMPANION PLATE TOTAL VOLUME (mL)	17.3	7.0	3.6