User Guide

Millicell® Cell Culture Inserts

6-, 12- and 24-well Hanging Membrane Inserts

for Cell Growth, Assays and Imaging

DOMINIQUE DUTSCHER SAS

Introduction

Millicell® Cell Culture Inserts are irradiated research devices promoting natural growth and differentiation of various cell types. These devices consist of tissue culture treated, track-etched polyethylene terephthalate (PET) membrane, sealed to a polystyrene housing.

The unique flange design suspends the membrane within a cell culture plate well, allowing cells to access media and nutrients from both their apical and basolateral sides. Gaps between the flanges facilitate pipetting between the outside of the insert and the cell culture plate well. The apical assist rim at the base of the insert allows for easier pipetting and media exchanges, protecting the cellular monolayer. These features support cell culture to more closely mimic *in vivo* cellular environment with improved growth, structure, differentiation, and function.

Our portfolio offers translucent and clear PET track-etched membranes of various pore sizes and well sizes to support user flexibility in biological assays. The translucent and high pore density membranes allow for increased rates of basolateral diffusion of nutrients to cells. The optically clear and low pore density membranes support imaging and microscopy capabilities.

Usage Guidelines

- For research use only.
- Store product at room temperature.
- Do not use if blister packaging is damaged.
- Handle inserts under aseptic conditions.
- Prior to use, inspect inserts and membrane to ensure they are undamaged.
- Do not use inserts above 37 °C when performing cell-based assays.
- Use compatible cell culture plates with well heights >15 mm.
- Do not use product with strong acids, strong bases, or organic solvents. For chemical compatibility data, go to SigmaAldrich.com/FilterChemicalCompatibility.

Specifications

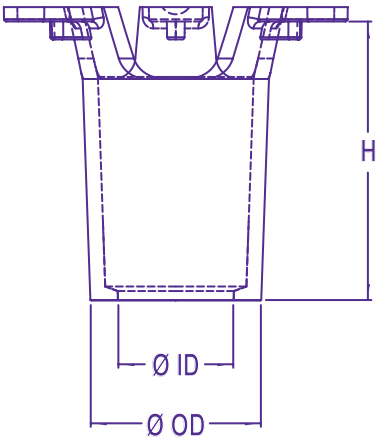
Materials of Construction

Membrane	Tissue culture treated, track-etched polyethylene terephthalate (PET), 9-16 µm thickness
Housing	Polystyrene

Properties

Gamma Irradiated

Insert Dimensions



	6-well	12-well	24-well
Height (H)	15.5 mm	15.5 mm	15.5 mm
Outer diameter (OD)	27 mm	15 mm	9 mm
Inner diameter (ID)	24 mm	12 mm	6.5 mm
Membrane area (effective)	4.5 cm^2	1.1 cm^2	0.3 cm^2

Procedure

Insert Handling

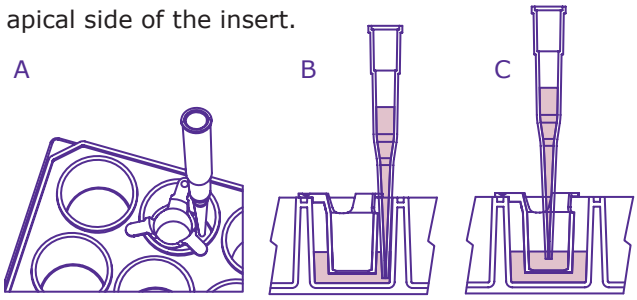
1. Select the appropriate cell culture insert based on pore size, optical clarity, and application. Refer to the online selection table as a guide when selecting insert.
2. Under aseptic conditions, peel the blister lid from the blister packaging and handle insert with a sterile forceps at the flanges. Inspect inserts and membrane are undamaged. Be careful not to touch the membrane.
3. Place insert in a compatible cell culture plate, ensure the flanges are flat and directly resting on the top of the well. The cell culture plate should be a minimum depth of 15 mm. We recommend Greiner Bio-One CELLSTAR® Cell Culture Multiwell Plates.



Cell Seeding

Refer to Media Volumes on page 3 for recommended media volumes.

1. Add appropriate pre-warmed cell culture media to basolateral side of the insert. Allow several minutes for the membrane in the insert to become moistened with media.
2. Prepare seeding solution by calculating appropriate seeding density to recommended working apical volumes.
3. Seed cells in pre-warmed cell culture media into the apical side of the insert.

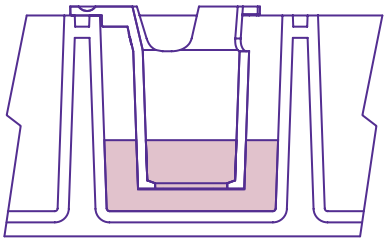


Easily access the basolateral side between the flanges (A) and aliquot basolateral volume (B). Then, fill apical side with appropriate cell seeding solution (C).

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Media Volumes

Cell culture plates may vary by size and depth of well. For optimizing volumes of a specific well plate, the meniscus of the apical volume in the insert should be leveled with the meniscus of the basolateral volume (right). This ensures equal pressure between both sides of the membrane.



We recommend Greiner Bio-One CELLSTAR® Cell Culture Multiwell Plates. Below are suggested volumes, highlighted volumes are optimal for cell seeding and growth.

Media Volumes Using Greiner Bio-One CELLSTAR® Cell Culture Multiwell Plates

The recommended volume is highlighted.

Plate Size	Volume	ALI* (mL)	Cell Seeding and Growth (mL)			
6-well	Apical	0	1.0	2.0	3.0	4.0
	Basolateral	1.0	1.8	2.8	3.6	4.2
12-well	Apical	0	0.2	0.4	0.6	0.8
	Basolateral	0.8	1.0	1.2	1.4	1.8
24-well	Apical	0	0.1	0.2	0.3	0.4
	Basolateral	0.3	0.5	0.7	1.0	1.2

*Air liquid interface

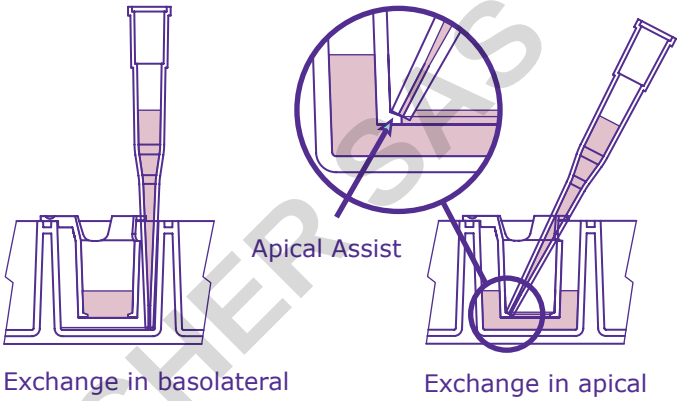
Media Volume in General Multiwell Plates During Standard Cell Seeding and Growth

The optimal volumes for plates with >15 mm depth is highlighted below.

Plate Size	Volume	Cell Seeding and Growth (mL)			
6-well	Apical	1.0	2.0	3.0	4.0
	Basolateral	2.0	2.8	3.5	4.3
12-well	Apical	0.2	0.4	0.6	0.8
	Basolateral	0.9	1.2	1.5	1.8
24-well	Apical	0.1	0.2	0.3	0.4
	Basolateral	0.6	0.9	1.2	1.5

Cell Growth and Feeding

Follow standard tissue culture incubation and feeding procedures for cell growth and monolayer formation. We recommend refreshing cell culture media every 2-3 days to ensure optimal cellular growth. Use the apical assist rim while performing media exchange to prevent membrane puncture and disruption to cells (below).



Note:

- Inserts can be used with or without extracellular matrix coating. This is dependent upon cell type.
- Avoid stacking plates containing inserts in the incubator. Any slight tilting may may result in uneven cell growth.
- With optically clear membrane inserts, cell growth can be monitored under a brightfield or phase contrast microscope.

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Assay Methods

Barrier Assays

Cellular barrier assays assess transendothelial and transepithelial permeability in various tissue models. Cellular barriers protect tissues and regulate the passage of a variety of biological molecules. Millicell® cell culture inserts provide a robust permeable support for cell growth and proliferation, supporting common barrier assays such as transepithelial-transepithelial electrical resistance (TEER) measurements and lucifer yellow assay. Optimize seeding density and media exchange protocols to ensure proper cellular monolayer formation.

For detailed information, procedures and sample data, please refer to our technical article at sigmaaldrich.com/barrier-formation-permeability-assays.

Note: For TEER experiments position the longer probe in the basolateral side while carefully placing the shorter probe in the apical side, avoid direct contact with cellular monolayer. Ensure TEER sensors are covered in cell culture media for stable readings.

Migration and Invasion Assays

Cellular migration and invasion assays evaluate movement of cells in response to a chemo-attractant or mechanical stimuli. It is critical to many facets of biology including development, immune response, tumor cell migration and invasion, and wound healing response. Millicell® inserts are a convenient permeable support to closely mimic these intrinsic processes for adherent and suspension cells. Users can utilize published protocols and literature to determine pore size, seeding density, and chemo-attractant for optimal signal to noise ratio during migration and invasion assays.

For detailed information, procedures and sample data, please refer to our technical article at sigmaaldrich.com/cell-migration-assay-guidance.

Microscopy: Fixing, Permeabilizing, and Staining

Imaging of the PET membrane can be utilized to observe membrane integrity, differentiated cellular markers, or specific cellular features. Millicell® inserts with optically clear membranes can be imaged directly from the insert while the translucent membrane can be excised and place on a slide. Inserts can be fixed with standard fixation and permeabilization techniques. Then, stained with small-molecule fluorescent stains or antibody-based techniques with no background fluorescence from PET membrane.

For detailed information, procedures and sample data, please refer to our technical article at sigmaaldrich.com/cellular-fluorescence-imaging.

Note:

- Clear membranes can be imaged directly from the insert. For optimal imaging, use a glass well bottom plate during imaging or sit the hanging insert in a larger well plate. Bringing the insert closer to the bottom of a well plate allows for a clearer image.
- Translucent membranes can be removed from housing with a #11 scalpel, slowly excise membrane at the outer circular edge of the housing. While a #13 scalpel can be used to trim or to divide membrane into separate pieces for staining.

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Product Ordering

To order, go to SigmaAldrich.com.

Millicell® Cell Culture Inserts

With PET membrane, individually blister packaged and sold in packs of 48

Pore Size (µm)	Pore Density (pores/cm²)	Optical Clarity	6-well Cat. No.	12-well Cat. No.	24-well Cat. No.
0.4	100 x 10^6	Translucent	PTHT06H48	PTHT12H48	PTHT24H48
	4 x 10^6	Clear	PCHT06H48	PCHT12H48	PCHT24H48
1.0	22 x 10^6	Translucent	PLRP06H48	PLRP12H48	PLRP24H48
	2 x 10^6	Clear	PTRP06H48	PTRP12H48	PTRP24H48
3.0	2 x 10^6	Translucent	PTSP06H48	PTSP12H48	PTSP24H48
	0.6 x 10^6	Clear	PCSP06H48	PCSP12H48	PCSP24H48
5.0	0.6 x 10^6	Translucent	PTMP06H48	PTMP12H48	PTMP24H48
8.0	0.2 x 10^6	Translucent	PTEP06H48	PTEP12H48	PTEP24H48
	0.06 x 10^6	Clear	PCEP06H48	PCEP12H48	PCEP24H48

Related Products

Description	Catalogue Number
Greiner CELLSTAR® 6-well culture plates	M8562
Greiner CELLSTAR® 12-well culture plates	M8687
Greiner CELLSTAR® 24-well culture plates	M8812
Millicell® ERS 3.0 Digital Voltohmmeter	MERS03000
Millicell® ERS 3.0 Standard Adjustable Electrode (for 6-, 12-, 24-well plates)	MERS03SAP
Millicell® Digital Cell Imager	MDCI10000
Scepter® 3.0 Handheld Automated Cell Counter	PHCC360KIT
Filter Forceps, blunt end, stainless steel	XX6200006P
Stericup®-GP Filter, PES membrane	S2GPU05RE
Millex®-GP Filter, PES membrane, sterile	SLGPR33RS
Steriflip®-GP Filter, PES membrane	SCGP00525
DAPI, for nucleic acid staining	D9542
Lucifer Yellow CH dipotassium salt	L0144
Crystal Violet	C0775

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