

User Guide

Millicell® Hanging Cell Culture Inserts

PTEP06H48
PTEP12H48
PTEP24H48
PTHT06H48

PTHT12H48
PTHT24H48
PTMP06H48
PTMP12H48

PTMP24H48
PTRP06H48
PTRP12H48
PTRP24H48

PTSP06H48
PTSP12H48
PTSP24H48

Introduction

Millicell® Hanging Cell Culture Inserts are general purpose devices for the growth and differentiation of various cell types.

In plastic tissue culture plates, cells can access media only from their apical sides, but in Millicell® tissue culture inserts, cells can access media from both their apical and basolateral sides. As a result, cell growth, structure, and function more closely mimic what occurs in vivo.

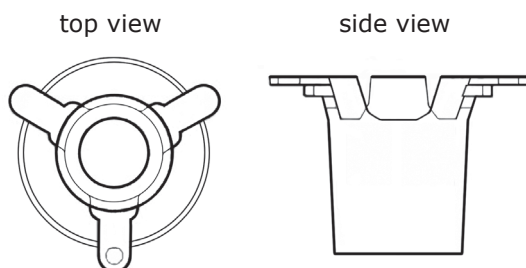
The insert's flanged design suspends it within the culture plate well and makes it possible to access both sides of the cell monolayer. Spaces between three protruding arms facilitate pipetting between the outside of the insert and the culture plate well.

Millicell® inserts are available to fit 6-, 12- and 24-well culture plates.

Usage Guidelines

- For research use only.
- Do not use if packaging is damaged.
- Before use, inspect inserts for membrane damage such as cracks or holes.
- Perform the following procedure in a laminar flow hood or equivalent controlled environment.
- Do not use inserts above 37 °C when performing cell based assays.
- Do not reuse inserts.
- Use culture plates with well heights > 15 mm.
- Do not use with strong acids, strong bases, or organic solvents.
- For chemical compatibility data, go to SigmaAldrich.com/FilterChemicalCompatibility.

Diagram of Millicell® Hanging Cell Culture Insert



Procedure

1. Prepare enough wells for experiments and controls.
2. Peel cover sheet from blister package containing the Millicell® insert.
3. Use sterile forceps to remove the insert from the package and place it into a culture plate well. Do not touch the membrane. Repeat until desired number of wells have inserts.
4. Add tissue culture media to basolateral side of each well, based on the table below.
5. Allow several minutes for the membrane in each insert to become moistened with the tissue culture media.
6. Seed appropriate number of cells onto the inside of insert above the membrane.
7. Follow standard tissue culture incubation/feeding procedures for cell growth and monolayer formation.
8. For migration/invasion assays, make sure chemoattractant is added to basolateral side.

NOTE: Be careful not to puncture the membrane or disturb cultured cells during media addition or removal. When seeding cells check literature to ensure proper seeding. Over seeding can cause stacking of cells during monolayer formation, whereas under seeding can cause low number of cell migration during migration assay.

Media Volumes for Standard Plastic Culture Plates

Recommended volumes for standard well plates are highlighted.

| Insert | 24-Well | 12-Well | 6-Well |
|--|---------------------|---------------------|---------------------|
| Well diameter | 6.5 mm | 12 mm | 24 mm |
| Membrane surface area | 0.3 cm ² | 1.1 cm ² | 4.5 cm ² |
| Apical volume (µL) | 100 µL | 200 µL | 1000 µL |
| | 200 µL | 400 µL | 2000 µL |
| | 300 µL | 600 µL | 3000 µL |
| | 400 µL | 800 µL | 4000 µL |
| Basolateral volume for Millicell® plates | 600 µL | 900 µL | 2000 µL |
| | 900 µL | 1200 µL | 2750 µL |
| | 1200 µL | 1500 µL | 3500 µL |
| | 1500 µL | 1800 µL | 4300 µL |

Specifications

Insert Dimensions

| | 24-well | 12-well | 6-well |
|---------------------------|---------------------|---------------------|---------------------|
| Height | 16 mm | 16 mm | 16 mm |
| Outer diameter | 9 mm | 15 mm | 27 mm |
| Inner diameter | 6.5 mm | 12 mm | 24 mm |
| Membrane area (effective) | 0.3 cm ² | 1.1 cm ² | 4.5 cm ² |

Membrane Specifications

| Pore Size (µm) | Pore Density (pores/cm ²) | Thickness (µm) | Optical Property |
|----------------|---------------------------------------|----------------|------------------|
| 0.4 | 1 × 10 ⁸ | 9-16 | Translucent |
| 1.0 | 2 × 10 ⁶ | 9-16 | Transparent |
| 3.0 | 2 × 10 ⁶ | 9-16 | Translucent |
| 5.0 | 6 × 10 ⁵ | 9-16 | Translucent |
| 8.0 | 2 × 10 ⁵ | 9-16 | Translucent |

Materials of Construction

Membrane: Polyethylene terephthalate (PET)
Plastic Holder: Polystyrene

Solvent Compatibility

Incompatible with strong acids, strong bases, and organic solvents. For more information, go to [SigmaAldrich.com/FilterChemicalCompatibility](https://www.sigmaaldrich.com/FilterChemicalCompatibility).

Properties

The devices are tissue culture treated and gamma irradiated, and ready for use as received. Extracellular matrix coating (ECM) is not required.

Product Ordering

Millicell® inserts are individually blister packed.
To order, go to SigmaAldrich.com.

Hanging Insert with translucent membranes**

| Pore Size | Qty/Pk | Cat. No. |
|-----------------------------------|--------|-----------|
| Millicell® 24-well Inserts | | |
| 0.4 µm | 48 | PTHT24H48 |
| 1.0 µm | 48 | PTRP24H48 |
| 3.0 µm | 48 | PTSP24H48 |
| 5.0 µm | 48 | PTMP24H48 |
| 8.0 µm | 48 | PTEP24H48 |
| Millicell® 12-well Inserts | | |
| 0.4 µm | 48 | PTHT12H48 |
| 1.0 µm | 48 | PTRP12H48 |
| 3.0 µm | 48 | PTSP12H48 |
| 5.0 µm | 48 | PTMP12H48 |
| 8.0 µm | 48 | PTEP12H48 |
| Millicell® 6-well Inserts | | |
| 0.4 µm | 48 | PTHT06H48 |
| 1.0 µm | 48 | PTRP06H48 |
| 3.0 µm | 48 | PTSP06H48 |
| 5.0 µm | 48 | PTMP06H48 |
| 8.0 µm | 48 | PTEP06H48 |

** With exception of 1.0 µm pore membrane inserts.

Related Products

| | Qty/pk | Cat. No. |
|---|--------|-----------|
| Millicell®-ERS Electrical Resistance System (measures membrane potential and resistance of epithelial cells in culture) | 1 | MERS00002 |
| Stericup®-GP Filter, PES membrane | 12 | S2GPU05RE |
| Millex®-GP Filter, PES membrane, sterile | 50 | SLGP033RS |
| Steriflip®-GP Filter, PES membrane | 25 | SCGP00525 |

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