

25 Tips and Techniques for Transwell® Permeable Supports from Corning

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Transwell Permeable Supports have been a lab standard for decades. Over that time, we've accumulated numerous tips and techniques for getting the most out of these versatile cell culture tools. Below are 25 of our favorites.

1. When changing the media for drug transport assays, always aspirate from the basolateral side first, then the apical side. When replacing the media, add in the opposite order (apical then basolateral).
2. Add liquid to 24-well inserts more easily by using every other tip on a multiple channel pipettor.
3. When seeding cells for migration assays, more is not always better. Optimization of the seeding concentration for each cell type is critical. Guidelines for MCF-7 and HT-1080 cells can be found in the *Cell Migration, Chemotaxis and Invasion Assay Protocol* (Corning CLS-AN-061).
4. When seeding cells on the underside of inserts, flip the entire plate upside down to allow the inserts to sit on the lid of the plate. Add cells as a single drop to the center of the insert and cover the majority of the membrane surface. After the cells have attached, replace the plate over the inserts and invert again. See *Seeding Cells onto the Underside of Transwell Permeable Supports* (Corning CLS-AN-533).
5. The receiver plates that come with the 96 HTS Transwell insert product are untreated. Treated receiver plates are available in clear, white and black.
6. Polyester (PET) inserts offer the best cell visualization properties.
7. Cells requiring extracellular matrix coatings on plastic substrates will also require them on permeable supports. Several protocols are available. Contact Corning for more information.
8. The 96 HTS Transwell inserts have access ports adjacent to Column One to allow aspiration and media addition to feeder reservoirs without having to separate the insert plate from the reservoir.
9. More consistent TEER measurement can be achieved when running drug transport applications by allowing plates to equilibrate to room temperature before measuring.
10. Crystal violet is a very easy and effective fixative/stain for enumerating cell migration when using Transwell® inserts.
11. When performing drug transport assays, more consistent results can be achieved by optimizing the passage number of the cells being used. For in-house MDCK cell studies, for example, the Corning Applications group uses cells between passages 11 and 25.
12. Transwell 6.5 mm inserts are sold with 12 inserts per plate. The 12 remaining wells allow for further insert manipulation to be conducted during assays after cell culture is complete.
13. When using the swabbing and staining method for enumerating migration or invasion, swab non-migrated cells prior to fixation and staining.



14. To image cells on the polycarbonate surface, fix and stain cells with any of the following stains: Crystal violet, Papanicolaou, Hematoxylin, Giemsa, May-Grunwald, or Wright's.
15. To help optimize cell growth on inserts, do not allow cell cultures to exceed 85% to 90% confluence on the cultureware that is to be used for seeding on the inserts.
16. To avoid damaging the cell culture surface, take care not to allow pipette tips to come in contact with the fragile membrane when removing or adding cells or medium to the insert.
17. Use care when transporting inserts to the incubator after cell seeding. Avoid spilling medium from the insert into the receiver plate, which will carry suspended cells into the receiver plate.
18. To speed up cell attachment to the membrane, try pre-soaking the inserts in cell growth medium containing serum proteins prior to cell seeding.
19. It is critical to use the proper Falcon® receiver plate with FluoroBlok™ insert systems. The FluoroBlok inserts (individual format) should be used with Falcon Cell Culture Insert Companion plates (24-well, Cat. No. 353504) to ensure proper positioning of the inserts in the wells. The plates supplied with FluoroBlok HTS 24 and 96 Multiwell inserts must be used for running the assay, labeling and reading samples to achieve reliable results.
20. To avoid swabbing and counting non-migrating cells in migration/invasion assays, consider using a dissociation solution to collect migrated cells in the receiver plate followed by fluorescent staining and reading. To further simplify the protocol, use FluoroBlok light blocking membranes and detect migrated cells directly on the membrane with no swabbing or cell dissociation.
21. Always check the underside of inserts for bubbles after addition of chemoattractant (or stain for FluoroBlok inserts) as they can interfere with cell growth and counting.
22. When using FluoroBlok inserts, always verify your plate reader results visually (i.e., using an inverted fluorescent microscope).
23. HUVECs and other endothelial cells demonstrate a bell curve response to growth factors such as VEGF. If their concentration is too high or too low, they may yield similar responses.
24. The endothelial cell source (HUVEC vs. HMVEC, etc.) can affect levels of protein expression and subsequent response to chemoattractants.
25. The FluoroBlok membrane exhibits negligible autofluorescence across the visible spectrum (400 to 700 nm) as demonstrated by top-reading fluorescence data. However, there is a low level of fluorescence background in bottom reading mode due to autofluorescence of, and/or reflection from, the polystyrene well bottom of the base plate.
Use of excessively high gain settings or failure to run the appropriate controls can often give the false impression that the FluoroBlok membrane blocks light inefficiently or has high inherent autofluorescence.

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

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