Corning® FluoroBlok™ Inserts Frequently Asked Questions

CORNING

We have made improvements to the original (purple) Corning FluoroBlok membrane. The new Corning FluoroBlok membrane is black and has improved spectral characteristics. General information in this FAQ applies to both versions, but specific wavelength ranges apply to the new (black) version.

For details, see Technical Bulletin CLS-DL-CC-042, *New PET Membrane for Corning FluoroBlok 3.0 µm and 8.0 µm Pore Size Cell Culture Inserts*.

Corning FluoroBlok inserts have a dyed polyethylene terephthalate (PET) microporous membrane that blocks light transmission at visible wavelengths between 400 to 700 nm. Migration assays are performed in the traditional manner using this insert system, with cells that are labeled via a fluorescent dye. This allows researchers to specifically detect and quantify fluorescently labeled cells that have migrated through the insert using a fluorescence plate reader or inverted fluorescence microscope.

Corning FluoroBlok inserts can be used to study a wide range of cell types and activities such as:

- Inflammation with neutrophils,[1-5] transepithelial[6] and transendothelial[7] migration; and analysis of blood-brain barrier,[8,9] dendritic cells,[10] and Macrophages[11]
- Pathways for stem cell differentiation[12,13]
- Screening for population-specific neuronal motogens[14]
- Migration of normal, transformed and transfected cells[15,16]
- Chemoinvasion assays, drug discovery[17]

Q: Can I do tumor cell invasion with Corning FluoroBlok inserts?

A. Yes, we have a wide variety of products for tumor cell invasion.

Corning BioCoat™ Tumor Invasion System 8.0 µm

Cat. No.	Description	Qty/Cs
354165	One insert plate with 24 well plate and lid	1
354166	Five insert plates with 24 well plates and lids	5
354167	One insert plate with 96 well plate and lid	1
354168	Five insert plates with 96 well plates and lids	5

For more technical information, visit our website at **www.corning.com/lifesciences**.

Q: Can I study Angiogenesis cell migration and invasion with the Corning FluoroBlok inserts?

A: Yes, we have a wide variety of products for Angiogenesis cell migration and invasion.

Corning BioCoat Angiogenesis System: Endothelial Cell Migration 3.0 µm

Cat. No.	Description	Qty/Cs
354143	One insert plate with 24 well plate and lid	1
354144	Five insert plates with 24 well plates and lids	5
351147	One insert plate with 96 well plate and lid	1
351148	Five insert plates with 96 well plates and lids	5

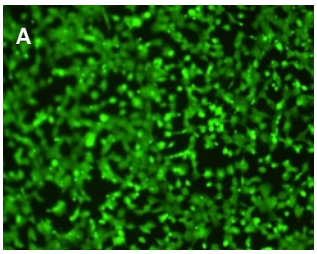
Corning BioCoat Angiogenesis System: Endothelial Cell Invasion 3.0 µm

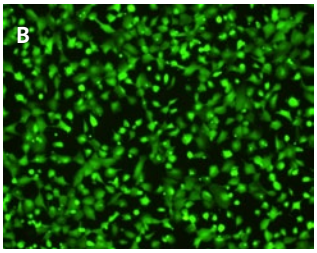
Cat. No.	Description	Qty/Cs
354141	One insert plate with 24 well plate and lid	1
354142	Five insert plates with 24 well plates and lids	5

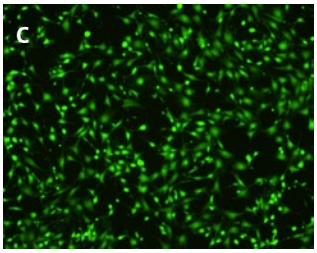
For more technical information, visit our website at **www.corning.com/lifesciences**.

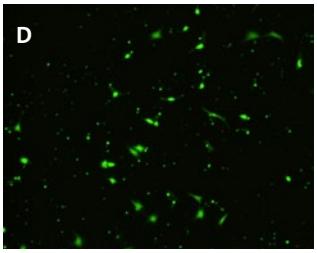
Tumor Invasion System - Model Assay

HT-1080 and 3T3 cells post-labeled with Calcein AM after migration through uncoated 8.0 micron inserts (fig A and C) and invasion through Corning Matrigel® coated 8.0 micron inserts (fig B and D). HT-1080 cells are capable of migration (A) and invasion (B). Lacking matrix metalloproteinases (MMPs), 3T3 cells can migrate (C) but not invade (D) through the basement membrane.









Q: Does the Corning® FluoroBlok™ membrane autofluoresce?

A: The Corning 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 Corning FluoroBlok membrane blocks light inefficiently or has high inherent autofluorescence.

Q: What is the advantage of using a Corning FluoroBlok insert instead of a typical clear cell insert?

A: Corning FluoroBlok inserts allow researchers to detect fluorescently labeled cells passing through the membrane in a homogeneous format (i.e., no further cell separation, washing or harvesting is necessary to detect cells specifically passing through the membrane). Simply add cells, stain (if necessary) and read cells on a bottom-reading plate reader.

Q: Why would I want to use a homogeneous assay with Corning FluoroBlok inserts?

A: Many cell migration and invasion assays require destructive, time-consuming manual processing of inserts for cell quantitation. Examples include using clear inserts for cell migration studies (cells must be removed from the lower well and labeled in some way for analysis) and invasion studies (cells are removed from the top side of the membrane with a cotton swab, and cells on the underside are then counted). With Corning FluoroBlok inserts, fluorescently labeled cells can be quantified directly and in real time with a fluorescence plate reader by reading the desired well for fluorescence output. For invasion and migration assays, no tedious manual sample processing is necessary, so assays may be automated for high throughput screening.

Q: How do Corning FluoroBlok inserts work?

A: The membrane within the insert blocks light transmission from 400 to 700 nm. By labeling cells with a fluorophore that has either excitation or emission wavelength within this range, it is possible to specifically quantify migration or invasion below the membrane. This can be done by exciting the well from below while simultaneously measuring fluorescence emission from below the membrane. The membrane prevents cells above the membrane from becoming excited or emitting and influencing the signal measured from below.

Q: I notice that when I look through the Corning FluoroBlok membrane, it does not appear to be opaque. How can it block light if I can see through it?

A: You are seeing light passing through the pores in the membrane. This is normal.

Q: What types of fluorescent dyes can I use to label cells and detect them with the Corning FluoroBlok insert?

A: Any dye that has an emission wavelength between 400 and 700 nm can be used with this system with high confidence. Outside of this spectral range, there will be more of a contribution from cells remaining in the apical chamber. Corning Calcein AM Fluorescent Dye (Cat. Nos. 354216 and 354217) and Corning $DiIC_{12}(3)$ Fluorescent Dye (Cat. No. 354218), are available for labeling cells. For information about these, and other compatible dyes, see our website **www.corning.com/lifesciences**.

Q: Are the Corning FluoroBlok inserts compatible with any plate?

A: No. It is critical to use the proper Falcon® receiver plate with the proper Corning FluoroBlok insert system. Individual inserts must be used with Falcon Cell Culture Insert Companion plates (24 well, Cat. No. 353504) to properly position the inserts in the wells. Corning FluoroBlok 24 Multiwell inserts use the supplied Falcon 24 well plates. These plates accurately fit the Corning FluoroBlok 24 Multiwell insert. The Corning FluoroBlok 96 Multiwell inserts use the supplied square well receiver plates. These plates must be used for running the assay, labeling and reading samples to achieve reliable assay results.

Q: Can Corning® FluoroBlok™ inserts be coated?

A: If desired, the inserts can be coated using Corning ECM proteins, such as Corning Matrigel® matrix for cell invasion, or Collagens and Fibronectin for migration. Uncoated Corning FluoroBlok inserts are available in a variety of formats and pore sizes:

Corning FluoroBlok Cell Culture Inserts

Cat. No.	Description	Qty/CS
351151	3.0 µm inserts for 24 well plates	48
351152	8.0 µm inserts for 24 well plates	48
Corning FluoroBlok 24-Multiwell Insert Systems 3.0 µm		
351155	One insert plate with 24 well plate and lid	1
351156	Five insert plates with 24 well plates and lids	5
Corning FluoroBlok 24-Multiwell Insert Systems 8.0 µm		
351157	One insert plate with 24 well plate and lid	1
351158	Five insert plates with 24 well plates and lids	5
Corning FluoroBlok 96-Multiwell Insert Systems 3.0 µm		
351161	One insert plate with 96 well plate and lid	1
351162	Five insert plates with 96 well plates and lids	5
Corning FluoroBlok 96-Multiwell Insert Systems 8.0 µm		
351163	One insert plate with 96 well plate and lid	1
351164	Five insert plates with 96 well plates and lids	5

If you need help selecting a pre-coated insert or ECM for your application, or need assistance coating your Corning FluoroBlok inserts, please contact Technical Support at 800.492.1110 or email CLSTechServ@corning.com.

Q: How can I be sure that the dye won't leach out of the membrane and contaminate my sample?

A: New formulation Corning FluoroBlok (black) membranes and insert systems were tested using commonly used biological solvents including: saline, 10% DMSO in culture medium, 4% paraformaldehyde, and 100% methanol. The effects on total transmission were negligible, and no observable dye was leached from the membranes.

Q: When I set up my plate reader, can I use standard 24 well and 96 well templates?

A: No, it is critical that the detector is properly positioned under the Corning FluoroBlok inserts. Consult our website **www.corning.com/lifesciences** for proper set up of your plate reader. If your plate reader is not listed, contact Technical Support at **800.492.1110** or email CLSTechServ@corning.com.

Q: Can I use Corning FluoroBlok inserts for the migration/invasion of suspension cells such as lymphocytes?

A: Yes, the inserts work well for non-adherent quickly migrating cells. If you pre-label your cells, you can collect kinetic data. Refer to our website at **www.corning.com/lifesciences** and the references listed.[1-11]

Q: Can cells be removed from the inserts after migration/invasion?

A: Yes. If the cells are adherent, use of trypsin, Corning Dispase (Cat. No. 354235), Corning Cell Recovery Solution (Cat. No. 354253), Accutase® or other enzymatic methods may prove successful.

References

1. Nick JA, et al. Recombinant human activated protein C reduces human endotoxininduced pulmonary inflammation via inhibition of neutrophil chemotaxis Blood 104(13):3878 (2004).
2. Kuijpers TW, et al. Apoptotic neutrophils in the circulation of patients with glycogen storage disease type 1b (GSD1b) Blood 101(12):5021 (2003).
3. Kuijpers TW, et al. Neutrophils in Barth syndrome (BTHS) avidly bind annexin-V in the absence of apoptosis Blood 103(10):3915 (2004).

4. Slofstra SH, et al. Inhalation of activated protein C inhibits endotoxin-induced pulmonary inflammation in mice independent of neutrophil recruitment Br J Pharmacol. 149(6):740 (2006).
5. Stegenga ME, et al. Effect of acute hyperglycaemia and/or hyperinsulinaemia on proinflammatory gene expression, cytokine production and neutrophil function in humans Diabet Med. 25(2):157 (2008).
6. Lin Ada, et al. Streptolysin S Inhibits Neutrophil Recruitment during the Early Stages of Streptococcus pyogenes Infection and Immunity 77(11):5190 (2009).
7. Johnson Louise A, et al. An inflammation-induced mechanism for leukocyte transmigration across lymphatic vessel endothelium JEM 203(12):2763 (2006).
8. Chaudhuri Anathbandhu, STAT1 signaling modulates HIV-1-induced inflammatory responses and leukocyte transmigration across the blood-brain barrier Blood 111(4):2062 (2008).
9. Chaudhuri Anathbandhu, 1 HIV-1 activates proinflammatory and interferon-inducible genes in human brain microvascular endothelial cells: putative mechanisms of blood–brain barrier dysfunction Journal of Cerebral Blood Flow & Metabolism 28:697 (2008).
10. Lapteva Natalia, et al. Attraction and Activation of Dendritic Cells at the Site of Tumor Elicits Potent Antitumor Immunity Molecular Therapy 17(9):1626 (2009).
11. Link Tiffany M, et al. TRPV2 has a pivotal role in macrophage particle binding and phagocytosis Nature Immunology 11:232 (2010).
12. Brown MD, et al. Influence of omega-6 PUFA arachidonic acid and bone marrow adipocytes on metastatic spread from prostate cancer British Journal of Cancer 102:403 (2010).
13. De Becker Ann, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3 Haematologica 92(4):440 (2007).
14. Hassoun Amani T, et al. A rapid screening method for population-specific neuronal motogens, substrates and associated signaling pathways Journal of Neuroscience Methods 166:178 (2007).
15. Bernhagen Jürgen, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment Nature Medicine 13:587 (2007).
16. Vitari Alberto C, et al. COP1 is a tumour suppressor that causes degradation of ETS transcription factors Nature Published online 15 May 2011.
17. Albini A, Noonan DM. The “chemoinvasion” assay, 25 years and still going strong: the use of reconstituted basement membranes to study cell invasion and angiogenesis. Curr Opin Cell Biol. 22(5):677-89 (2010).

To place an order in the U.S., contact Customer Service at:
tel: **800.492.1110**, fax: 978.442.2476
email: **CLSCustServ@corning.com**

For technical assistance, contact Technical Support at:
tel: **800.492.1110**, fax: 978.442.2476
e:mail **CLSTechServ@corning.com**

Outside the U.S., contact your local distributor or visit **www.corning.com/lifesciences** to locate your nearest Corning office. For additional Corning product, technical, or distributor information, call **978.442.2200**.

CORNING

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

© 2013 Corning Incorporated Printed in U.S.A. 12/13 CLS-DL-CC-027 REV2