Corning® FluoroBlok™ Inserts

Frequently Asked Questions

CORNING

The Corning FluoroBlok membrane is black and has improved spectral characteristics. General information in this FAQ applies to both black and former purple versions, but specific wavelength ranges apply to the black version.

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

Corning FluoroBlok inserts have a black 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 an 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 screening[17, 18]

1. Can I do tumor cell invasion with Corning FluoroBlok inserts?

Yes. You can coat the FluoroBlok inserts with Corning Basement Membrane matrix, just as you would a clear insert.

Visit www.corning.com/lifesciences to access a protocol for Cell Invasion Assay (CLS-DL-CC-031) as a guide for self-coating the membrane.

2. Can I study angiogenesis cell migration and invasion with the Corning FluoroBlok inserts?

Yes. The following product can be used to study angiogenesis cell migration using FluoroBlok inserts.

Corning BioCoat Angiogenesis System: Endothelial Cell Migration 3.0 µm

Cat. No.	Description	Qty/Cs
354144	Five insert plates with 24-well plates and lids	5

3. Does the Corning FluoroBlok membrane autofluoresce?

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.

4. **What is the advantage of using a Corning® FluoroBlok™ insert instead of a typical clear cell insert?**

   FluoroBlok inserts allow researchers to detect fluorescently labeled cells, or cells transfected to express fluorescent proteins, 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.

5. **Why would I want to use a homogeneous assay with Corning FluoroBlok inserts?**

   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 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.

6. **How do Corning FluoroBlok inserts work?**

   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.

7. **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?**

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

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

   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, visit www.corning.com/lifesciences.

9. **Are the Corning FluoroBlok inserts compatible with any plate?**

   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.

10. **Can Corning FluoroBlok inserts be coated?**

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

Products may not be available in all markets.

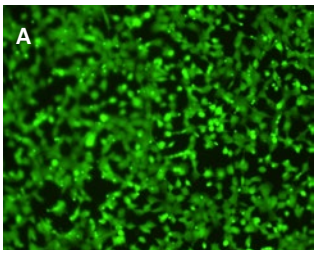
Corning FluoroBlok Cell Culture Inserts

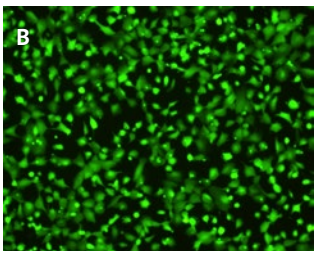
Cat. No.	Description	Qty/CS
351151	3.0 µm inserts for 24-well plates	48
351152	8.0 µm inserts for 24-well plates	48

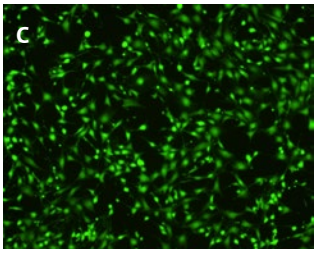
Corning FluoroBlok 24-Multiwell Insert Systems, 3.0 µm

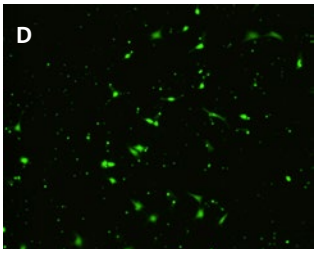
Tumor Invasion Model Assay

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









Cat. No.	Description	Qty/CS
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

For assistance selecting a pre-coated insert or ECM for your application, or coating your Corning FluoroBlok inserts, contact ScientificSupport@corning.com.

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

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.

12. When I set up my plate reader, can I use standard 24-well and 96-well templates?

No. It is critical that the detector is properly positioned under the Corning FluoroBlok inserts. Visit www.corning.com/lifesciences for proper set up of your plate reader. If your plate reader is not listed, contact ScientificSupport@corning.com.

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

Yes. The inserts work well for non-adherent quickly migrating cells. If you pre-label your cells, you can collect kinetic data. Visit www.corning.com/lifesciences and the references listed.[1-11]

14. Can cells be removed from the inserts after migration/invasion?

Yes. If the cells are adherent, use of trypsin, Corning Dispase (Cat. No. 354235), Corning Cell Recovery Solution (Cat. No. 354253), Accutase® (Cat. No. 25-058-CI) or other enzymatic methods may prove successful. Refer to Corning Matrigel® Matrix Frequently Asked Questions (CLS-DL-CC-026) at www.corning.com/lifesciences to see which cell dissociation reagents to use based on downstream applications.

References

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