

BD Falcon FluoroBlok Inserts

Frequently Asked Questions

BD Falcon™ FluoroBlok™ inserts have a dyed polyethylene terephthalate (PET) microporous membrane that blocks light transmission at visible wavelengths between 490–700 nm. Migration assays are performed in the traditional manner using this insert system, with cells that are labeled using 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.

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

- Inflammation with neutrophils¹⁻⁵, transepithelial⁶ and transendothelial⁷ migration; and analysis of blood-brain barrier⁸⁻⁹, dendritic cells¹⁰, and Macrophages¹¹
- Pathways for stem cell differentiation^{12,13}
- Screening for population-specific neuronal motogens¹⁴
- Migration of normal, transformed and transfected cells^{15,16}

Can I do tumor cell invasion with BD FluoroBlok inserts?

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

Cat. No.	Description	Qty.
BD BioCoat™ Tumor Invasion System 8.0 µm		
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: bdbiosciences.com

Can I study Angiogenesis cell migration and invasion with the BD FluoroBlok inserts?

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

Cat. No.	Description	Qty.
BD BioCoat Angiogenesis System: Endothelial Cell Migration 3.0 µm		
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

Cat. No.	Description	Qty.
BD BioCoat Angiogenesis System: Endothelial Cell Invasion 3.0 µm		
354141	one insert plate with 24-well plate and lid	1
354142	five insert plates with 24-well plates and lids	5

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Does the BD FluoroBlok membrane autofluoresce?

Yes. Normal PET clear membranes do demonstrate some level of autofluorescence; however, the dyes incorporated into the BD FluoroBlok membrane quench any noticeable autofluorescence.

What is the advantage of using a BD FluoroBlok insert instead of a typical clear cell insert?

BD FluoroBlok inserts allow researchers to detect fluorescently tagged cells passing through the membrane in a "homogeneous" format (ie, no further cell separation, washing or harvesting is necessary to detect cells specifically passing through the membrane).

Migration of Calcein AM Labeled Human Microvascular Endothelial Cells through BD Falcon FluoroBlok 24-Multiwell Insert System



- Serum



+ Serum

Human microvascular endothelial cells (HMVECs) labeled with the fluorescent dye Calcein AM were placed in a BD Falcon FluoroBlok 24-Multiwell Insert System (3.0 μm pore size) in either the absence (-Serum) or presence (+10% Serum) of chemoattractant. Cells were visualized using an Olympus IMT-2 phase epifluorescent microscope. Images were captured using IPWIN 4.0 software.

Why would I want to use a "homogeneous" assay with BD Falcon FluoroBlok inserts?

Many cell migration and invasion assays require destructive, time-consuming manual processing of inserts for cell quantitation. Examples include cell migration studies (cells must be removed from the lower well and labeled in some way for analysis) and invasion studies (cells are removed off the top side of the membrane with a cotton swab and cells on the underside are then counted). With BD Falcon™ FluoroBlok™ inserts, fluorescently labeled cells can be quantified directly and in real time by using a fluorescence plate reader and 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.

How do BD Falcon FluoroBlok inserts work?

The BD FluoroBlok membrane within the BD Falcon FluoroBlok insert blocks light transmission from 490-700 nm. By labeling cells with a fluorophore that has both excitation and emission wavelengths 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 exclusive BD FluoroBlok membrane prevents cells above the membrane from becoming excited and contaminating the signal measured from below.

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

The human eye can detect wavelengths from 400–650 nm. The BD FluoroBlok membrane can pass light from 400–490 nm in the visible range. It is these blue wavelengths that the human eye can see through the insert. For light transmission in the range of 490–700 nm, the BD FluoroBlok membrane will block >99.5% of the photons passing through.

What types of fluorescent dyes can I use to label cells and detect them with the BD Falcon FluoroBlok insert?

Fluorescent dyes derived from the fluorescein, rhodamine and cyanine families can all be used with this system, as long as their emission wavelengths are within 490–700 nm. BD™ Fluorescent Dyes, BD™ Calcein AM Fluorescent Dyes (Cat. Nos. 354216 and 354217) and BD™ DiIC₁₂(3) Fluorescent Dye (Cat. No. 354218), are available for labeling cells. For information about these, and other compatible dyes, See BD Technical Bulletin 451 on our website: bdbiosciences.com

Are the BD Falcon FluoroBlok inserts compatible with any plate?

No. It is critical to use the proper BD Falcon receiver plate with the proper BD FluoroBlok insert system. The BD Falcon FluoroBlok inserts (individual format) must be used with BD Falcon Cell Culture Insert Companion plates (24-well, Cat. No. 353504) to properly position the inserts in the wells. BD Falcon FluoroBlok 24-Multiwell inserts use the supplied BD Falcon 24-well plates. These plates accurately fit the BD Falcon FluoroBlok 24-Multiwell insert. The BD Falcon 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.

Can BD FluoroBlok inserts be coated?

If desired, BD FluoroBlok™ inserts can be coated using BD™ ECM proteins such as BD Matrigel™ matrix for cell invasion, or BD Collagens and BD Fibronectin for migration. BD Falcon™ FluoroBlok inserts (uncoated) are available in a variety of formats and pore sizes:

Cat. No.	Description	Qty.
BD Falcon FluoroBlok Cell Culture Inserts		
351151	3.0 µm inserts for 24-well plates	48
351152	8.0 µm inserts for 24-well plates	48
BD Falcon 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
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
BD Falcon 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
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 BD FluoroBlok inserts, please contact Technical Support at 877.232.8995 or email labware@bd.com

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

Our testing has shown that the dye can only leach out if the membrane is treated with strong organic solvents. These same conditions would also kill cells. Aqueous solvents and buffers will not leach detectable quantities of the dye during routine use.

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 BD FluoroBlok inserts. Consult BD Technical Bulletin 436 on our website (bdbiosciences.com) for proper set up of your plate reader. If your plate reader is not listed, contact Technical Support at 877.232.8995 or email labware@bd.com

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

Yes, BD FluoroBlok inserts work well for non-adherent quickly migrating cells. If you pre-label your cells, you can collect kinetic data. Other methods to quantify migration using non-adherent cells are radiolabeling and collecting cells for manual counting. Neither allows kinetic data without using separate inserts for each time point. See BD Technical Bulletin 457 "Optimized Chemotaxis Conditions for Primary Blood Monocytes or THP-1 Cells Using BD Falcon™ FluoroBlok™ 96-Multiwell insert plates" on our website: bdbiosciences.com and the references listed¹⁻¹¹.

References

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