Corning® BioCoat™ Matrigel® Invasion Chambers

Frequently Asked Questions

CORNING

The Corning BioCoat Matrigel Invasion Chamber is a useful tool to study the invasion of malignant cells. For cells to metastasize, they must be able to secrete proteases that break down the basement membrane as well as migrate. Invasion through a Corning Matrigel matrix-coated cell culture insert has become the gold standard for low throughput quantitative and qualitative measurement of cellular metastatic potential, including investigation of the mechanisms of cell invasion and inhibition of cell invasion[1-10].

Q: How do I prepare Corning BioCoat Matrigel invasion chambers for use?

A: Remove the package from storage at -20°C and allow it to come to room temperature.

Confirm that the inserts are in the notches of the companion plates. Add warm bicarbonate-based media to the interior of the inserts. For exact volumes, see *Corning BioCoat Matrigel Invasion Chamber Guidelines for Use* (enclosed with the product). Allow to rehydrate for two hours in a 5% CO_2 incubator.

NOTE: If you do not want to use all of the inserts in the same experiment, do not allow them to thaw, as repeated freeze-thaws will damage the Matrigel matrix barrier. Open the package under aseptic conditions, and, using sterile forceps, transfer the inserts to a separate companion plate to thaw. Wrap the unused inserts in the original packaging and quickly return them to the -20°C freezer.

Q: Should I add chemoattractant into the upper or lower chambers?

A: The chemoattractant should be placed in the lower, or basolateral, chamber. It slowly diffuses into the upper, or apical, chamber setting up a chemotactic gradient for the cells to follow. See Corning Biocoat Matrigel Invasion Chamber Guidelines for use for detailed instructions.

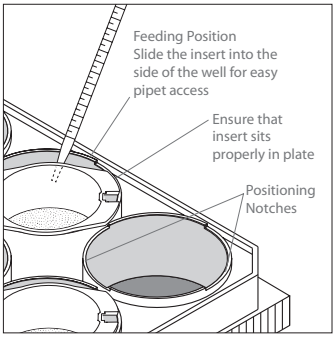
Air Bubble

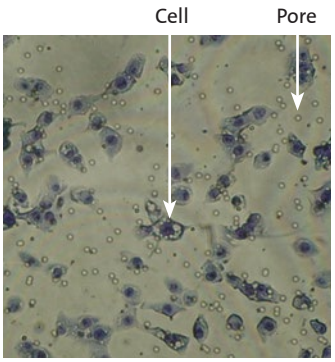
Q: Does it matter if I have air bubbles, and how do I prevent them?

A: It is critical that the BioCoat Matrigel invasion chambers are in the notches and there are no air bubbles. Add pre-warmed media containing chemoattractant to the wells of the Falcon® tissue culture companion plate, or add just media to the negative control wells. Use sterile forceps to transfer the chambers into the previously filled wells of the companion plate. To avoid air bubbles, tip the chamber at a slight angle as it is lowered into the liquid and align the notches. If there are air bubbles, carefully tap the side of the plate to dislodge the bubbles.

Q: I don't want cells to be weak during the migration/invasion assay, so can I add serum to the upper chambers?

A: We do not recommend adding serum to the apical chamber; try using 0.1% BSA instead. We recommend starting with bovine serum albumin (Corning Cat. No. 354331). If you must add serum to the apical compartment, use 0.4%. High concentrations of serum in the apical compartment interfere with the chemotactic gradient.





Q: I used Corning® BioCoat™ Matrigel® invasion chambers and the Corning BioCoat control inserts, but the cells were uneven on the bottom of the insert. Why did this happen?

A: There will be some normal variability, and you should count multiple fields. All conditions should be run in triplicate. Always check for air bubbles when setting up your assay, because cells will not migrate through the dry patches. Always use Falcon® 24 well cell culture insert companion plates that come with the system (Corning Cat. No. 353504) and Falcon 6 well cell culture insert companion plates (Corning Cat. No. 353502)—do not use standard multiwell plates. Make sure the inserts are in the positioning notches of the companion plates.

Q: What will the cells look like under the microscope?

A: Cells will look slightly different depending on the cell type. The cells will have an irregular shape. The pores will be round or ovoid and be refractile.

Q: If incubation times are long, do cells proliferate after migration/invasion?

A: We recommend doing a migration control on uncoated inserts and calculating the percent invasion by dividing the number of cells that invaded through the Corning Matrigel invasion chamber by the number of cells that migrated through the control insert and multiplying by 100. This controls for proliferation. See the *Corning BioCoat Matrigel Invasion Chamber Guidelines* for use for details.

Q: What reagent do you recommend for staining the cells on the membrane?

A: We recommend any colorimetric stain that differentially stains the cytoplasm and the nucleus such as hematoxylin and eosin (H&E) or Wrights stain. Two examples of these types of stains are Diff-Quik™ (VWR Cat. No. 47733-150) and Hemacolor® Stain Set (Merck KGaA, Darmstadt, Germany, Cat. No. 65044-93).

Q: Are there any tips to wipe off the Matrigel matrix from the insert?

A: You should remove the liquid from the top of the insert and pre-wet the cotton swab. Hold the insert at an angle so the bottom of the membrane is not touching a flat surface and gently swab out the gel, taking care to remove the cells but not detaching the insert membrane from the housing.

Q: How can I remove the membrane? Do you supply a special blade?

A: Do not remove the membrane from the housing until the staining is complete. A special blade is not supplied. Use a #11 scalpel to cut the membrane from the housing and immediately mount cell-side down on a microscope slide. Put a drop of immersion oil on the membrane, and cover it with a coverslip.

Q: How long can I keep the hydrated insert? Is it possible to store for several days after the hydration?

A: You should use the rehydrated insert after the normal 2-hour rehydration period. If you must use it the next day because your cells are not ready expect the CV between replicates to increase. Do not use after 24 hours.

Q: I did not get very much invasion of my cells, what should I do?

A: Verify, with an inverted microscope, that the invaded cells have not detached from the bottom of the insert and have fallen to the bottom well.

Since there is no serum in the seeding media, make sure you either use a non-enzymatic method of removing the cells prior to seeding, or do a serum wash to deactivate the trypsin. Make sure you are using the notched Falcon® 24 well cell culture insert companion plates (Corning Cat. No. 353504) and Falcon 6 well cell culture insert companion plate (Corning Cat. No. 353502) and that the cell culture inserts are in the notches. Verify there are no air bubbles trapped under the inserts.

Different cell types invade at different rates, and the protocol may need to be adjusted for different cell types. As a first step, increase the serum concentration to 10% in the lower chamber and increase the cell number to 50,000 cells per 24 well insert or 250,000 cells per 6 well insert. If this does not increase the raw invasion numbers sufficiently, you can increase the invasion time from 24 to 48 hours.

The Corning BioCoat Matrigel invasion chamber is highly cited in the scientific literature with specific details for many cell types. You can contact Corning Life Sciences Technical Support for assistance in locating a reference for your specific cell type.

Q: I am tired of counting all of those inserts for my IC_{50} experiments, is there an easier way to quantify invasion?

A: Yes, we have the Corning® BioCoat™ 24 Multiwell (Corning Cat. Nos. 354165 one pack; 354166 five pack) and 96 Multiwell (Corning Cat. Nos. 354167 one pack; 354168 five pack) tumor cell invasion systems. These systems utilize Corning FluoroBlok™ membranes, a patented light-tight membrane that efficiently blocks the transmission of light within the range of 400 to 700 nm which allows fluorescence detection in a simplified and non-destructive manner. Fluorescently labeled cells present in the top chamber of the insert are made invisible to a bottom-reading fluorescence plate reader by the FluoroBlok membrane. Once labeled cells migrate through the membrane they are no longer shielded from the light source and are easily detected with a bottom-reading fluorescence plate reader. The BioCoat tumor invasion systems can be used for either kinetic or end-point invasion assays.

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