

Corning® BioCoat™ Tumor Cell Invasion Systems

Frequently Asked Questions

CORNING



Corning BioCoat Tumor Cell Invasion Systems are available in 24- or 96-Multiwell permeable support plate formats. Both contain the proprietary light-tight Corning FluoroBlok™ PET membrane (8.0 µm pore size) that is specifically designed to block the transmission of light from 400 to 700 nm. The Corning FluoroBlok membrane is uniformly coated with Corning Matrigel® matrix that serves as an authentic reconstituted basement membrane *in vitro*. This provides a true barrier to non-invasive cells while presenting an appropriate protein structure for invasive cells. Fluorescently labeled cells present in the apical compartment are shielded from bottom-reading fluorescence plate readers, allowing detection of invaded cells in the basolateral compartment without the need for manual steps such as cell scraping, manual counting, or dye extraction.

The Corning BioCoat Tumor Cell Invasion Systems have been used to study *in vitro* cell invasion with a large number of cell types, in many different ways, including but not limited to:

- ▶ Using microRNA to study invasion inhibition in gastric cancer¹
- ▶ Inhibition of bladder cancer invasion, but not prostate cancer invasion, with Valproic acid²
- ▶ ERBB2 up-regulating metastatic phenotype in medulloblastoma using Davoy cells³
- ▶ Evodine's pathway-specific impact on human lung adenocarcinoma invasion using H1299 cells⁴
- ▶ Gap junctional intercellular communication and expression of connexins on metastatic potential of MD-MBA-231 cells⁵
- ▶ Impact of inducing COX-2 expression on invasion, in MD-MBA-231 and MCF-7 cells⁶
- ▶ Impact of zinc on invasion of PC-3 cells through NF-kB and AP-1 transcription factors⁷

1. How many cells should I seed? What should I use as a chemoattractant? How long should I run the assay?

Corning has several documents that you may consult to help determine this, including the Corning BioCoat Tumor Invasion System Guidelines for Use.

Additionally, Application Note 484 – Migration of Human Mesenchymal Stem Cells Using Corning FluoroBlok Cell Culture Inserts (CLS-DL-CC-054) and Technical Bulletin 457 – Optimized Chemotaxis Conditions for Primary Blood Monocytes or THP-1 Cells Using Corning FluoroBlok 96-Multiwell Inserts Plates (CLS-DL-CC-078) provide examples of migration/chemotaxis assays using FluoroBlok Inserts that contain this information. These documents can be found at www.corning.com/lifesciences.

Ultimately, you may need to optimize these conditions for your specific cell type of interest. Application Note – Considerations when Optimizing your Chemotaxis or Invasion Assay (CLS-AN-188) – provides some general guidelines for how to optimize these variables.

2. I'm currently using the Corning Matrigel Invasion Chamber and it is working well, but it is a slow and manual process. My optimized cell concentration, chemoattractant concentration, and time of assay are different from the recommended protocol for the Corning BioCoat Tumor Invasion System, what should I do?

If you have an optimized cell number, chemoattractant, and assay period for invasion using the Corning Matrigel Invasion Chamber, you should use the same conditions for the Corning BioCoat Tumor Cell Invasion System. The cell number will need to be scaled if you are currently using the 24-well invasion chamber and plan to scale up to the 96-well invasion chamber. Contact Scientific Support if you need help with scaling or any other aspect of the protocol.

3. Is there anything unusual or distinct when using the Corning® BioCoat™ Tumor Cell Invasion System compared to the Corning Matrigel® Invasion Chamber?

Yes, the Corning BioCoat Tumor Cell Invasion System is based on the Corning FluoroBlok™ membrane. Before starting your experiments, you should read the Corning FluoroBlok Frequently Asked Questions (CLS-DL-CC-027). In addition, you should set up your plate reader according to Technical Bulletin 436 – Set Up Guidelines and Dimensional Templates for Fluorescence Plate Readers Used With Corning FluoroBlok Permeable Support Systems and Corning BioCoat Multiwell Permeable Support Cell-Based Assays (CLS-DL-CC-074). Both of these documents are available at www.corning.com/lifesciences. If your plate reader is not listed on Technical Bulletin 436, contact Scientific Support for more information.

4. I followed the protocol as directed and my cells migrated very well, but I did not observe good invasion. Is there something wrong with the Corning BioCoat Tumor Cell Invasion System?

No, some metastatic cells are more invasive than others in an invasion assay. Technical Bulletin 442 – Screening of Anti-metastatic Compounds by a Fluorescence-based Tumor Cell Invasion Assay (CLS-DL-CC-076) has modified protocols for less invasive cell types. It is available on our website at www.corning.com/lifesciences. If your cell type is not listed, you can consult the scientific literature, or contact Scientific Support for more information.

5. The Corning BioCoat Tumor Invasion System was thawed and gelled, but I do not have enough cells for my experiment. What can I do? Do I have to throw out the plate?

We recommend using the plate immediately after rehydrating for two hours at 37°C in a humidified incubator. It is possible to rehydrate overnight, but the variation between replicates will increase.

6. Do I need to use the entire plate in one experiment?

Yes, it is critical to follow the thawing and rehydration procedure. If the plate is thawed and refrozen, the Corning Matrigel basement membrane matrix layer will be damaged.

7. What fluorescent dye should I use for an end-point assay? What is the best dye for a kinetic assay?

Calcein AM works very well for labeling in an end-point assay (post-labeling), since it is not fluorescent until cleaved by intracellular esterases. It is available in two sizes: 1 mg (Cat. No. 354217) and 0.5 mg [10 x 50 µg/vial] (Cat. No. 354216).

There are a number of dyes that work well for kinetic assays (pre-labeling cells at beginning of assay). Additional information about dyes for pre-labeling is available in Technical Bulletin 451 – Compatible Fluorophores and Dyes for Corning FluoroBlok Permeable Supports and Permeable Support Systems (CLS-DL-CC-077) at www.corning.com/lifesciences.

8. Do I need to run a migration control?

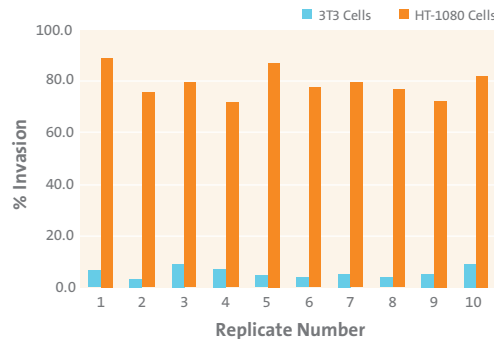
The migration controls allow you to normalize invasive potential between different cell lines by calculating an invasive index. Refer to the Corning BioCoat Tumor Invasion System Guidelines For Use for these calculations. Migration controls also allow you to test the impact of different treatments on migration.

Many researchers publish without running a migration control. If migration controls are not done, the data are presented as a percent or fold increase from the basal condition for treatments that increase invasion or as a percent decrease from the positive control for treatments that decrease (inhibit) invasion.

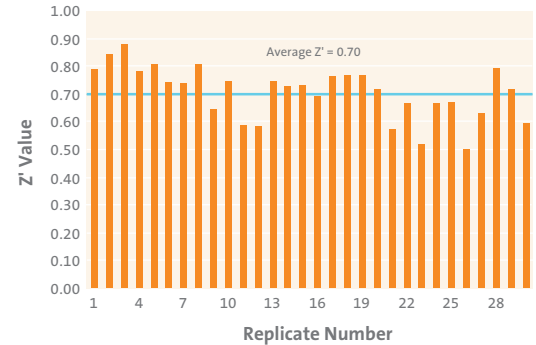
9. I observe high variability between replicates in my assay. How do I decrease this variability?

It is critical to properly set up the Fluorescence Plate Reader. If not, high variability, or low signal will result. You should set up your plate reader according to Technical Bulletin 436 – Set Up Guidelines and Dimensional Templates for Fluorescence Plate Readers Used With Corning® FluoroBlok™ Permeable Support Systems and Corning BioCoat™ Multiwell Permeable Support Cell-based Assays (CLS-DL-CC-074) at www.corning.com/lifesciences. If your plate reader is not listed on Technical Bulletin 436, contact Scientific Support for more information.

24-Multiwell

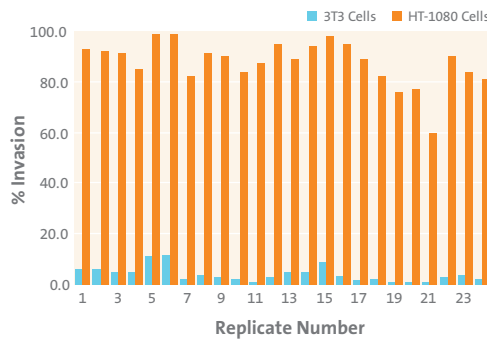


Comparison of Mean Percent Invasion. Multiple lots of the Corning BioCoat 24-Multiwell Tumor Invasion System were assayed. A CytoFluor® plate reader was then used to measure fluorescently labeled cells residing on the bottom of the permeable support membrane post-invasion. Mean percent invasion of NIH-3T3 and HT-1080 cells was compared. Cells were labeled post-invasion using Calcein AM.

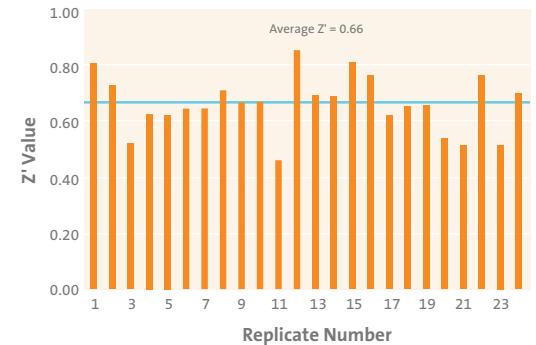


Z' Values for Tumor Cell Invasion. Using multiple lots of Corning BioCoat 24-Multiwell Tumor Invasion System, two operators ran ten separate assays with three plates from each assay. Z' was calculated using % invasion of HT-1080 cells and % invasion of NIH-3T3 cells (negative control). Cells were labeled post-invasion using Calcein AM.

96-Multiwell



Comparison of Mean Percent Invasion. Multiple lots of the Corning BioCoat 96-Multiwell Tumor Invasion System were assayed. A Victor2™ plate reader was used to measure fluorescently labeled cells on the bottom of the permeable support membrane post-invasion. Mean percent invasion of NIH-3T3 and HT-1080 cells was compared. Cells were labeled post-invasion using Calcein AM.



Z' Values for Tumor Cell Invasion. Using multiple lots of Corning BioCoat 96-Multiwell Tumor Invasion System, three operators ran eight separate assays with three plates from each assay. Z' was calculated using % invasion of HT-1080 cells and % invasion of NIH-3T3 cells (negative control). Cells were labeled post-invasion using Calcein AM.

10. If I have a cell type already transfected with GFP (Green Fluorescent Protein), will the cells need to be labeled?

If you have an efficient transfection, you should be able to run the assay without adding exogenous dye. A representative study with transfected tumor cells is found in Technical Bulletin 441 – Screening of Anti-metastatic Compounds Using ZsGreen1 Reef Coral Fluorescent Protein (RCFP) Labeled HT-1080 Tumor Cells (CLS-DL-CC-075) at www.corning.com/lifesciences.

11. How can I view a demonstration of the Corning® BioCoat™ Tumor Invasion System?

There is a video demonstrating this assay at www.jove.com/video/1475/an-in-vitro-fluoroblok-tumor-invasion-assay.

12. Can I use the Corning BioCoat Tumor Invasion System in a co-culture experiment?

We have not performed this assay, but Corning FluoroBlok™ permeable supports coated with Corning Matrigel® matrix have been used to study PC-3 cell invasion in a co-culture model with BMS (Bone Marrow Stroma) as a chemoattractant⁸.

References

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2. Chen CL, Valproic Acid Inhibits Invasiveness in Bladder Cancer but Not in Prostate Cancer Cells. *JPET* 319(2) 533-542 (2006).
3. Hernan R. ERBB2 Up-Regulates S100A4 and Several other Prometastatic Genes in Medulloblastoma. *Cancer Res.* 63:140-148 (2003).
4. Takada Y, et al. Evodiamine Abolishes Constitutive and Inducible NF-κB Activation by Inhibiting IκBα Kinase Activation, Thereby Suppressing NF-κB-regulated Antiapoptotic and Metastatic Gene Expression, Up-regulating Apoptosis, and Inhibiting Invasion. *J. Biol. Chem.* 280(17):17203 (2005).
5. Li Z, et al. Alterations in Cx43 and OB-cadherin affect breast cancer cell metastatic potential. *Clin. Exp. Metastasis* 25:265-272 (2008).
6. Larkins TL, et al. Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression. *BMC. Cancer* 6:181 (2006).
7. Uzzo RG, et al. Diverse effects of zinc on NF-κB and AP-1 transcription factors: implications for prostate cancer progression. *Carcinogenesis* 27(10):1980-1990 (2006).
8. 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: 403-413 (2010).

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

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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

Worldwide Support Offices

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