New PET Membrane for Corning® FluoroBlok™ 3.0 µm and 8.0 µm Pore Size Cell Culture Inserts

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Jeff Partridge, M.S., Stephen Rimsa, M.S., and Paula Flaherty, M.S.
Corning Incorporated
Life Sciences
Tewksbury, MA

Introduction

Corning FluoroBlok Cell Culture Inserts and Insert Systems now feature a new, enhanced source of material. An improved membrane was developed that provides the same great features as the original Corning FluoroBlok inserts but with enhanced light-blocking efficiency. While the majority of applications should yield similar output, optimization of some assays may be necessary.

Physical Specifications

The color of the membrane has changed from purple to black (see Fig. 1). Plate-based dimensions will remain the same as only the membrane has changed; insert frames and base plates are unaffected by this material change. (To set up your fluorescence plate reader with the correct plate map, see *Technical Bulletin Set-Up Guidelines and Dimensional Templates for Fluorescence Plate Readers used with Corning FluoroBlok Insert Systems and Corning BioCoat™ Multiwell Insert Cell-Based Assays,* CLS-DL-CC-074).

Figure 1. New Membrane Color

Previous color of membrane

New color of membrane

Light Blocking Efficiency

The new membranes allow less than 2% light transmission from 360 to 690 nm. This represents a two-fold reduction in light pass through (from 360 to 490 nm) when compared to the previous membrane. These enhancements permit the use of blue fluorescent dyes such as DAPI and the Hoechst stains for cell enumeration and multiplexing with increased confidence in results (see Figs. 2 and 5).

Cell Migration and Invasion

Corning FluoroBlok Inserts (8.0 µm pore size) were used in a model cell invasion assay. Invasive HT-1080 cells and non-invasive 3T3 cells (data not shown) were seeded in the apical chambers of both Corning Matrigel®-coated and uncoated inserts. Chemoattractant solution was added to the basal chambers of the system, and after overnight incubation, cells were stained with Corning Falcon® Calcein AM florescent dye, Corning Cat. No. 354216. Fluorescence was measured using a PerkinElmer EnVision® plate reader (see Figs. 3-5).

Results indicated no statistically significant differences in either cell migration or invasion between the previous and new membranes. Invasion indexes and Z' factors were comparable and consistent with previous results (ref. *Corning BioCoat™ Tumor Invasion System Guidelines for Use*, Corning Cat. No. 354165 and 354166).

3.0 µm membrane	**Previous**	**New**
Pore size	3 ± 0.3 µm	3 ± 0.3 µm
Pore density	$8 \pm 2 \times 10^5$ pores/cm^2	$8 \pm 2 \times 10^5$ pores/cm^2
Thickness	22 ± 3 µm	Same

8.0 µm membrane	**Previous**	**New**
Pore size	7.3 ±1 µm	7.3 ±1 µm
Pore density	1×10^5 pores/cm^2	$6 \pm 2 \times 10^4$ pores/cm^2
Thickness	18 ± 3 µm	18 ± 3 µm

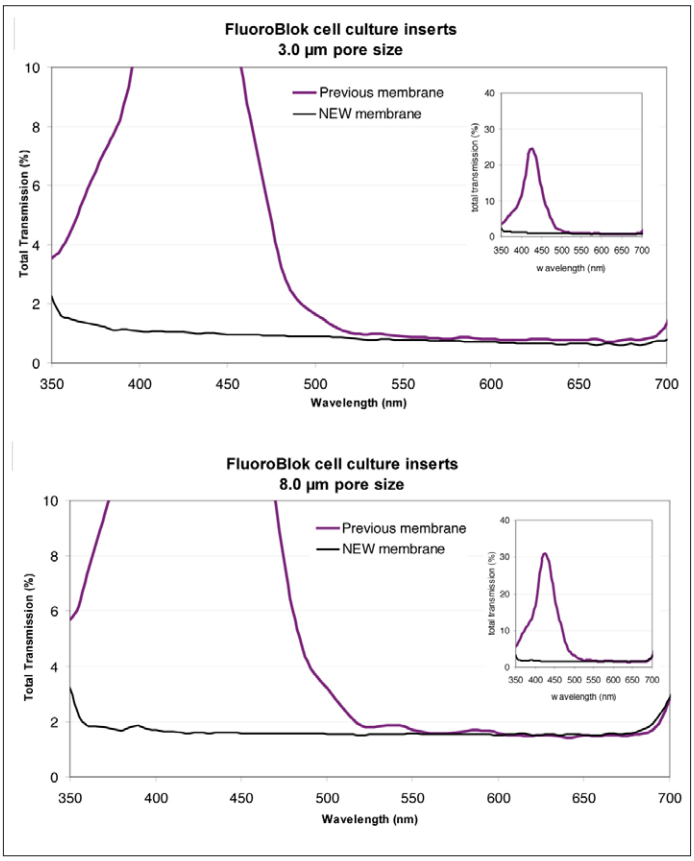

Figure 2. Total Transmission Spectra for 3.0 µm (top) and 8.0 µm (bottom) pore size Corning® FluoroBlok™ inserts as measured by transmission spectrophotometry using HunterLab UltraScan® PRO. Insets detail expanded y axis results.

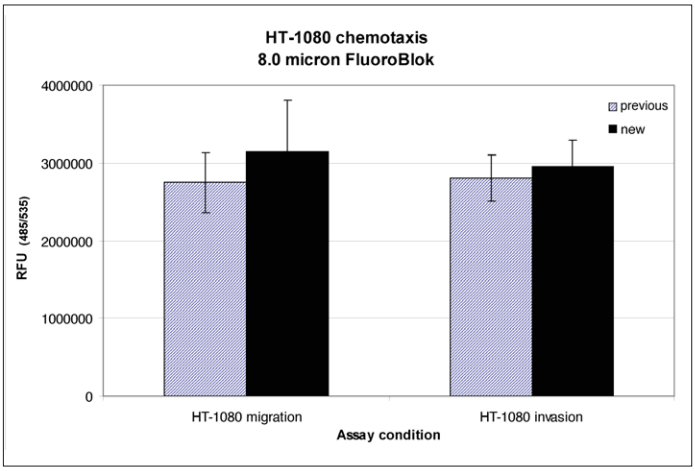

Figure 3. Migration (uncoated inserts) and invasion (Corning Matrigel®-coated inserts) of HT-1080 cells. The invasion index (invasive HT-1080 cells: non-invasive 3T3 cells) was 10.9 for the previous membrane and 10.1 for the new membrane. Z' factors (a statistical characteristic of assay suitability) were previous, 0.616; new, 0.558 (previous, n=6, new, n=24).

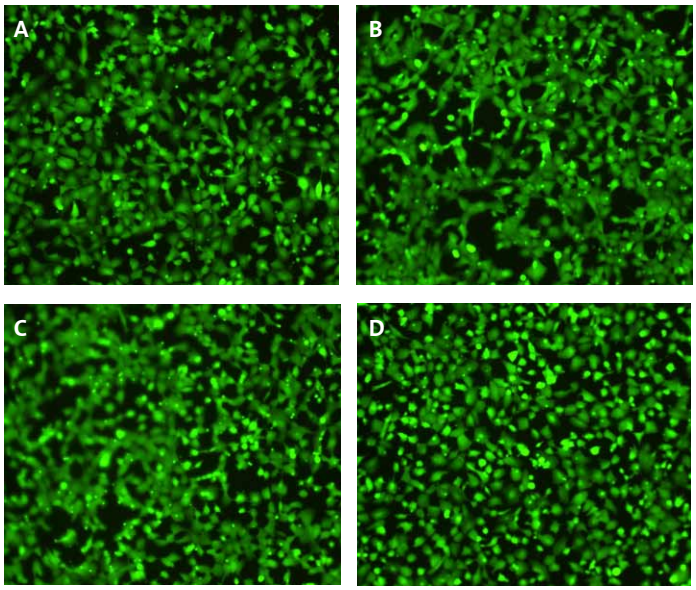

Figure 4. HT-1080 stained with calcein AM. (A) previous membrane – migration, (B) previous membrane – invasion, (C) new membrane – migration, (D) new membrane – invasion.

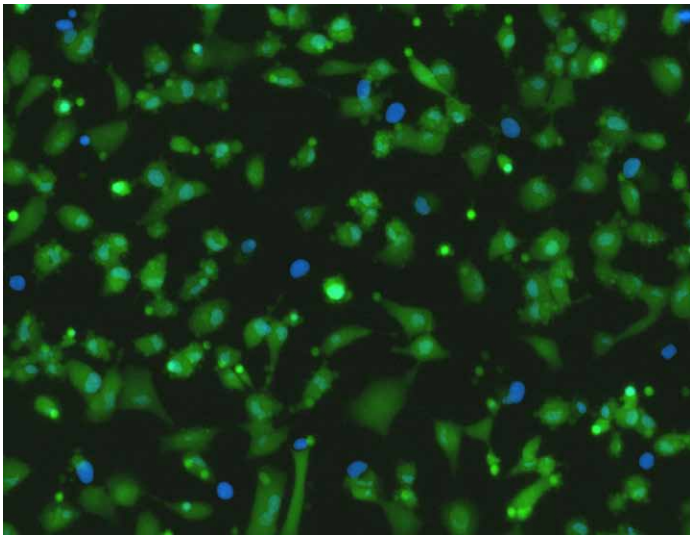

Figure 5. HT-1080 stained with Hoechst 33342 (blue) and calcein AM (green).

Affected Catalog Numbers

Cat. No.	Description	Qty/Pk	Qty/Cs
Corning® FluoroBlok™ Inserts and Insert Systems, High Density PET, 3.0 µm Pore Membrane			
351151	3.0 µm cell culture insert for 24 well plates	1	48
351155	24 multiwell system	1	1
351156	24 multiwell system	5	5
351161	96 multiwell system	1	1
351162	96 multiwell system	5	5
Corning FluoroBlok Inserts and Insert Systems, High Density PET, 8.0 µm Pore Membrane			
351152	8.0 µm cell culture insert for 24 well plates	1	48
351157	24 multiwell system	1	1
351158	24 multiwell system	5	5
351163	96 multiwell system	1	1
351164	96 multiwell system	5	5
Corning BioCoat™ Tumor Invasion Systems, 8.0 µm Pore Membrane			
354165	24 multiwell system	1	1
354166	24 multiwell system	1	5
354167	96 multiwell system	1	1
354168	96 multiwell system	1	5
Corning BioCoat Angiogenesis Systems, 3.0 µm Pore Membrane			
354141	3.0 µm pore PET membrane with Corning Matrigel® Matrix	1	1
354142	3.0 µm pore PET membrane with Corning Matrigel Matrix	1	5
Corning BioCoat Fibronectin Inserts and Angiogenesis Systems, 3.0 µm Pore Membrane			
354597	BioCoat Fibronectin Cell Culture Inserts, 3.0 µm, inserts in two 24 well plates		2
354143	Angiogenesis System for Endothelial Cell Migration, one insert plate with one 24 well plate and lid		1
354144	Angiogenesis System for Endothelial Cell Migration, five insert plates with five 24 well plates and lids		5
354147	Angiogenesis System for Endothelial Cell Migration, one insert plate with one 96 well plate and lid		1
354148	Angiogenesis System for Endothelial Cell Migration, five insert plates with five 96 well plates and lids		5

Bibliography

Zhang, J-H., Chung, TDY., Oldenburg, KR. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen (1999) 4:67.

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836 North St.
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Corning acquired the BioCoat,™ FluoroBlok™ and Matrigel® brands. For information, visit www.corning.com/discoverylabware.

Corning Incorporated, One Riverfront Plaza, Corning, NY 14831-0001