

The BD Falcon™ FluoroBlok™ 96-Multiwell Insert System Enhances High-Throughput Analysis of Cell-Based Assays

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Introduction

Cell-based assays on microporous membranes provide predictive *in vitro* model systems for drug discovery in many areas, such as angiogenesis, neoplasia, and inflammation. However, they have generally been impractical for use in high-throughput drug screening until recently due to the lack of automation-friendly formats, poor signal-to-noise, and time-consuming manual data analysis. Advances in fluorescence detection technology and the availability of a wide range of fluorophores for cell labeling have enabled rapid, non-destructive monitoring of cell migration. Such assays can now be performed in homogeneous fashion and increased throughput following the introduction of the BD Falcon™ FluoroBlok™ Insert Systems (BD Cat. No. 351161 or 351162).

Although the BD Falcon FluoroBlok 24-Multiwell Insert System can provide a significant increase in throughput over individual inserts, true miniaturization and high-throughput cannot be attained for these assays without a 96-well format. Available fluid handling equipment generally does not handle 24-well formats. Most screening groups avoid implementing assays in less than 96-well format for these reasons, relegating them to low-throughput, confirmative screens, or research applications. We report initial studies with the BD Falcon FluoroBlok 96-Multiwell Insert System, which show its performance in crosstalk and cell migration assays.

Materials and Methods

Cell Labeling

The HT-1080 human fibrosarcoma cell line was grown to 75-85% confluence in BD BioCoat™ 75 cm² flasks (Cat. No. 354462) using DMEM/10% FCS. The cells were then labeled *in situ* with 10 μM Calcein AM in DMEM for one hour at 37°C. Labeling with DiI at 10 μg/ml was carried out in DMEM/10% FCS for the same length of time. DMEM, FCS, Calcein AM and DiI were purchased from Invitrogen™ Corporation. After washing, the cell monolayer was briefly trypsinized to lift the cells, which were pelleted and resuspended in DMEM/0.1% BSA. Cells were 95-98% viable after labeling.

Calcein AM and DiI are now available from BD Biosciences.

BD™ Fluorescent Dyes

BD™ Fluorescent Dyes are available for labeling cells when performing tumor cell invasion, angiogenesis assays, and other cell-based assays. For additional information about BD™ Calcein AM Fluorescent Dye (Cat. Nos. 354216 and 354217) and BD™ DiI_{C₁₂(3)} Fluorescent Dye (Cat. No. 354218), visit our website at: bdbiosciences.com.

Cell Migration Assays

Cell migration assays were performed in BD Falcon FluoroBlok 96-Multiwell Insert plates with 8 μm PET membrane. The insert housing is made of black PET to block crosstalk and a 96-well receiver plate is optimized to accommodate the insert and side port. Cells were added to the upper chambers at the indicated densities in 50 μl of DMEM/BSA. Media with 5% FCS was used as a chemoattractant in the lower wells, while DMEM/0.1% BSA was added to the control wells (270 μl). The plates were incubated for four hours at 37°C, after which fluorescence

of cells which had migrated through the microporous membrane was measured on the Applied Biosystems CytoFluor® 4000 and PerkinElmer HTS 7000 Plus fluorescent plate readers using excitation/emission wavelengths of 485/530 nm for Calcein AM or 530/590 nm for DiI (Figure 2).

For comparison, cell migration assays were also performed in BD Falcon FluoroBlok 24-Multiwell Insert plates (Cat. No. 351185). Cells were added to the upper chamber at a density of 50,000 cells/insert.

To generate a standard curve, the cells were diluted in DMEM/BSA to give the indicated cell number in 100 μl and dispensed into BD Falcon 96-well plates (Cat. No. 353915). Fluorescence was quantitated as described above.

Crosstalk Measurements

Oregon Green® 514 and sulforhodamine 101 (Molecular Probes) were dissolved to 50 μM in PBS and an equal volume of each was mixed. For top-to-top crosstalk measurements, 50 μl of fluorophore solution was added to a BD Falcon FluoroBlok 96-Multiwell Insert in a checkerboard pattern. For bottom-to-bottom crosstalk analysis, 270 μl of fluorophore was added to the receiver plate in a checkerboard pattern and the remaining wells received an equal volume of PBS. Fluorescence was quantitated with the BD Falcon 96-Multiwell Insert placed on the Applied Biosystems CytoFluor 4000, PerkinElmer HTS 7000 Plus, or PerkinElmer Victor2™ using excitation/emission wavelengths of 485/530 nm for Oregon Green or 590/645 nm for sulforhodamine 101. Top-to-top crosstalk was measured in top-reading mode, while bottom-to-bottom crosstalk was measured in bottom-reading mode (Figure 1).

Conclusions

- Bottom-to-bottom crosstalk between the wells of the BD Falcon™ FluoroBlok™ 96-Multiwell Insert System was minimal, even when high amounts of fluorophore were used in the receiver plate. When measured with the CytoFluor® 4000, which has the largest detector, only 300 ppm of crosstalk was detected out of 84,000 counts of fluorophore. Top-to-top crosstalk and fluorescence blocking efficiency were also excellent (Figure 1).
- Cell migration assays could be easily scaled down with no loss in sensitivity. Starting with as few as 4000 Calcein AM or DiI labeled cells, cell migration could be detected in a four-hour assay. Real-time kinetics of assays could be determined, even with low input cell numbers (Figure 2).
- There was a linear relationship between the number of input cells and the number of cells migrating. This indicates that the number of pores in the small membrane area was not saturated, even at higher input cell numbers (Figure 3).
- Low variability was observed, with CVs in the range of 5-15% (Table 4).

A)					
Applied Biosystems		Net Mean FIU Results			Raw Mean
Cytofluor® 4000		Source	X-Talk	Control	Blank
Mean FIU	86,624	26.3	-0.1	17.1	
std - dev	1068	8.2	0.0	0.3	
CV %	1.23	31.3	0.0	1.47	
Crosstalk (%)	0.0304		-0.0001	485 nm	
Crosstalk (ppm)	304		-1	530 nm	
Oregon Green 514					
PerkinElmer		Net Mean FIU Results			Raw Mean
Victor2™		Source	X-Talk	Control	Blank
Mean FIU	1,645,651	48.4	11.1	342.9	
std - dev	27853	34.8	11.3	17.5	
CV %	1.69	71.8	102	5.10	
Crosstalk (%)	0.0029		0.0007	485 nm	
Crosstalk (ppm)	29		7	535 nm	
Oregon Green 514					
Applied Biosystems		Net Mean FIU Results			Raw Mean
Cytofluor® 4000		Source	X-Talk	Control	Blank
Mean FIU	85,007	23.0	0.3	66.5	
std - dev	1096	8.0	0.0	1.0	
CV %	1.29	35.0	0.0	383	
Crosstalk (%)	0.0270		0.0003	590 nm	
Crosstalk (ppm)	270		3	645 nm	
Sulforhodamine 101					
PerkinElmer		Net Mean FIU Results			Raw Mean
Victor2™		Source	X-Talk	Control	Blank
Mean FIU	671,781	17.4	-16.1	425.1	
std - dev	16398	24.2	21.4	28.0	
CV %	2.44	139	-133	6.58	
Crosstalk (%)	0.0026		-0.0024	580 nm	
Crosstalk (ppm)	26		-24	642 nm	
Sulforhodamine 101					
PerkinElmer		Net Mean FIU Results			Raw Mean
HTS 7000 Plus		Source	X-Talk	Control	Blank
Mean FIU	107,525	3.4	0.9	71.4	
std - dev	2397	8.6	5.0	9.2	
CV %	2.23	253	559	12.93	
Crosstalk (%)	0.0032		0.0008	485 nm	
Crosstalk (ppm)	32		8	530 nm	
Oregon Green 514					
Applied Biosystems		Net Mean FIU Results			Raw AFL
Cytofluor 4000		Source	X-Talk	Pre-Read	Pre-Read
Mean FIU	35,648	-8.0	1573		
std - dev	33860	63.9	53.7		
CV %	94.99	-801	3.42		
Crosstalk (%)	-0.0224			485 nm	
Crosstalk (ppm)	-224			530 nm	
Oregon Green 514					
Applied Biosystems		Net Mean FIU Results			Raw AFL
Cytofluor 4000		Source	X-Talk	Pre-Read	Pre-Read
Mean FIU	76,054	2.2	465		
std - dev	7414	13.3	8.6		
CV %	9.75	598	1.84		
Crosstalk (%)	0.0029			590 nm	
Crosstalk (ppm)	29			645 nm	
Sulforhodamine 101					

Figure 1: Crosstalk studies of the BD Falcon™ FluoroBlok™ 96-Multiwell Insert System, (A) bottom-to-bottom crosstalk; (B) top-to-top crosstalk.

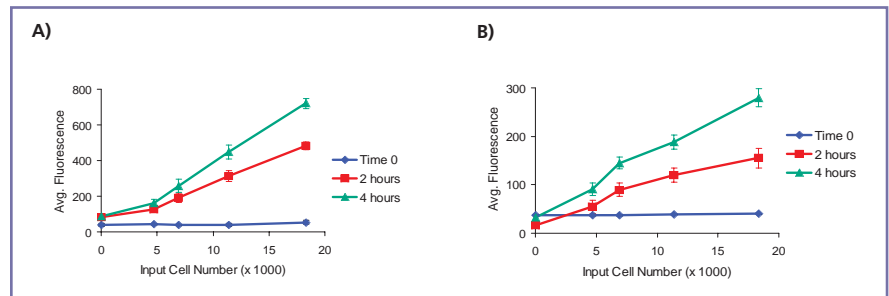


Figure 2: Migration of Calcein (A) and DiI (B) labeled HT-1080 cells through BD Falcon™ FluoroBlok™ 96-Multiwell Inserts, 8 μm pore size. Values represent the mean of 8 wells ± SD. Migration from as few as 4000 input cells can be detected.

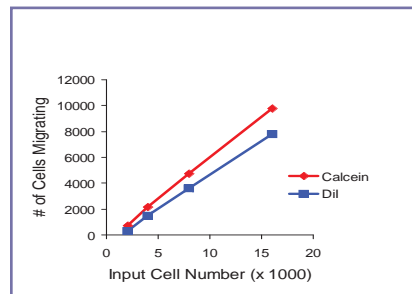


Figure 3: Relationship of number of cells migrated to input cell number. The four-hour fluorescence data from Figure 2 was converted to cells migrated using the standard curve. The relationship was linear at all input cell concentrations.

Calcein	Treatment	Cell #	CV %			
Control	Serum	8000	14.7			
		2000	11.5			
		4000	7.4			
		8000	5.3			
		16000	3.9			
DiI	Treatment	Cell #	CV %			
				Control	8000	7.3
				Serum	2000	6.7
					4000	7.5
					8000	12.3
		16000	4.2			

Table 4: Assays run in BD Falcon™ FluoroBlok™ 96-Multiwell Inserts demonstrate good variability. Values were calculated from n=8 wells.

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