

## Technical Bulletin #451

## Compatible Fluorophores and Dyes for BD Falcon™ FluoroBlok™ Inserts and

Compatible Fluorophores for BD Falcon™ FluoroBlok™ Inserts and Insert Systems				
Dye	Filter Set (λ Ex/λ Em)	Nature of Labeling	Intracellular Stability	Stock
BD™ Calcein AM Fluorescent Dye BD Cat. Nos. 354216 and 354217	485 nm/530 nm	Non-covalent, retained after intracellular ester cleavage	Short-term: 4-6 hours after labeling	5 mg/ml in DMSO* <sup>1</sup>
BD™ DiI Fluorescent Dye BD Cat. No. 354218	530 nm/560 nm	Non-covalent, incorporates into cell membrane	Long-term: 3-4 weeks in culture	5-10 mg/ml in DMSO <sup>1-3</sup>
DiO Molecular Probes (MP) Cat. No. D-1125	485 nm/530 nm	Non-covalent, incorporates into cell membrane	Long-term: 3-4 weeks in culture	0.3% (w/v) in 1:9 DMSO:99.5% etOH <sup>3</sup>
(5, 6) CFDA, SE MP Cat. No. C-1165 (5, 6) CFDA, SE ** MP Cat. No. C-1157	485 nm/530 nm	Covalent, modification of intracellular amino groups	Long-term: up to 8 weeks <i>in vivo</i>	10 nM in DMSO <sup>4</sup>

\*The final concentration of DMSO should be less than or equal to 0.2% (v/v) in labeling media  
\*\*The fluorescence of CFDA, SE is more sensitive than CFDA, SE to fluctuations in intracellular pH

## 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 Dyes (Cat. Nos. 354216 and 354217) and BD™ DiI<sub>12</sub>(3) Fluorescent Dye (Cat. No. 354218), visit our website at: [bdbiosciences.com](http://bdbiosciences.com).

## Labeling Protocols

All suggested labeling procedures may require optimization depending on cell type and application.

## 1. Calcein AM

Follow instructions from BD Calcein AM (Cat. Nos. 354216 and 354217) Guidelines for Use. Resuspend to stock concentration of 5 mg/ml in DMSO. Add to labeling media to give final concentration of 5-10 µg/ml. To minimize extracellular cleavage of calcein AM by serum proteases, serum-free labeling media should be used if possible. However, the HUVECs should be labeled in media containing 2% serum to enhance viability. Attached cells should be labeled *in situ*; 3.5-4 mls per BD Falcon™ 75 cm<sup>2</sup> flask is sufficient. After labeling for two hours at 37°C, wash the cells to remove any unincorporated dye and proceed with your assay. To label suspended cells, see *Reference 2*.

## 2. DiI and DiO

Follow instructions from BD DiI Fluorescent Dye (Cat. No. 354218) Guidelines for Use. For DiO, sonicate the stock for 10 minutes.<sup>3</sup> If DMSO stocks have been frozen in aliquots, thaw at 37°C and sonicate briefly. Attached cells can be labeled in growth media at 2-10 µg/ml overnight. Sterile filter the media containing dye before adding it to the cells. Wash cells thoroughly before proceeding with assay. Suspended cells can be labeled.<sup>1</sup> **Note:** PMNs cannot be labeled with DiI following dextran sedimentation and/or hypotonic RBC lysis; use Percoll™ (GE Healthcare Bio-Sciences) or Polymorphprep™ (AXIS-SHIELD PoC AS) instead.

## 3. (5, 6) CFDA, SE and CFDA, SE

Cells must be labeled in amine-free buffers such as PBS or HBSS, as the fluorophores will react with the amino acids in media. To prevent lifting of attached cells, additional Ca<sup>2+</sup>/Mg<sup>2+</sup> may be necessary. Dilute the stock solution to 2-10 µM and label the cells for 30 minutes at 37°C. Remove the dye and incubate in media for another 30 minutes.

## 4. CellTracker™ Probes

Dilute the stock solution to 1-10 µM in media such as DME. Incubate the cells for 45 minutes at 37°C. Remove the dye and incubate in media for another 30 minutes.

## Compatible Dyes for BD Falcon™ Fluoroblok™ Inserts and Insert Systems

This table can be used to choose dye markers with similar applications to replace markers that are outside the fluorescence blocking range of the BD Falcon™ Fluoroblok™ membrane.

Incompatible Marker	Application	λ (Ex)	λ (Em)	Compatible Marker	Application	λ (Ex)	λ (Em)
Fura-2	Ca <sup>2+</sup> probe, ratiometric	300-400 nm	510 nm	Fura Red™	Ca <sup>2+</sup> probe, ratiometric	472-436 nm	657-637 nm
Dansyl chloride	amine-reactive, conjugates	335 nm	518 nm	ATTO-TAG™ FQ ATTO-TAG CBQCA	amine-reactive, conjugates "	486 nm 465 nm	591 nm 560 nm
Lucifer yellow CH (hydrazide)	Polar tracer, impermeant	428 nm	536 nm	Sulforhodamine 101 Sulforhodamine B	Polar tracer, impermeant stain, impermeant	586 nm 565 nm	605 nm 586 nm
<b>Fluorophores</b>							
7-amino-4-methylcoumarin	conjugates and enz-substrates	353 nm	442 nm	4'-(aminomethyl)-fluorescein 5-(aminomethyl)-fluorescein BODIPY® 530/550 EDA BODIPY TR cadaverine Lissamine rhodamine B EDA Texas Red® cadaverine	conjugates and enz-substrates " " " " "	492 nm 492 nm 534 nm 588 nm 561 nm 591 nm	516 nm 516 nm 551 nm 616 nm 581 nm 612 nm
4-(Cl17)-7-hydroxycoumarin	pH indicator, membrane surface	366 nm	453 nm	Carboxy SNARF®-1 AM Carboxy SNARF®-1 diacetate	pH indicator, intracellular "	548-576 nm 508-540 nm	587-635 nm 543-623 nm
Cascade Blue® hydrazide	covalent stain, impermeant	399 nm	419 nm	Sulforhodamine 101 Sulforhodamine B	stain, impermeant "	586 nm 565 nm	605 nm 586 nm
Calcein Blue AM	Ca <sup>2+</sup> probe, fixed λ	322 nm	435 nm	BD™ Calcein AM (Cat. No. 354216 and 354217) Calcium Orange™ AM Calcium Crimson™ AM	Ca <sup>2+</sup> probe, fixed λ " "	494 nm 549 nm 589 nm	517 nm 576 nm 615 nm
<b>Fluorescent Cell-Binders</b>							
Hoechst 33258	DNA probe, cell-permeable	352 nm	461 nm	Hexidium iodide Dihydroethidium (w/redox) LD 5751 (MP Cat. No. L-75 95) Propidium iodide SYTOX® Green	DNA probe, cell-permeable " " DNA probe, cell-impermeant "	518 nm 518 nm 543 nm 535 nm 504 nm	600 nm 605 nm 712 nm 617 nm 523 nm
Hoechst 33342	DNA probe, cell-permeable	350 nm	461 nm	Hexidium iodide Dihydroethidium (w/redox) LD 5751 (MP Cat. No. L-75 95) Propidium iodide SYTOX Green	DNA probe, cell-permeable " " DNA probe, cell-impermeant "	518 nm 518 nm 543 nm 535 nm 504 nm	600 nm 605 nm 712 nm 617 nm 523 nm
DAPI	DNA probe, cell-permeable	358 nm	461 nm	Hexidium iodide Dihydroethidium (w/redox) LD 5751 (MP Cat. No. L-75 95) Propidium iodide SYTOX Green	DNA probe, cell-permeable " " DNA probe, cell-impermeant "	518 nm 518 nm 543 nm 535 nm 504 nm	600 nm 605 nm 712 nm 617 nm 523 nm
<b>Fluorescent DNA-Bind Derivatives</b>							
BOBO-1	DNA probe, cell-impermeant	462 nm	481 nm	BOBO™-3	DNA probe, cell-impermeant	570 nm	604 nm
POPO-1	DNA probe, cell-impermeant	434 nm	456 nm	POPO™-3	DNA probe, cell-impermeant	534 nm	570 nm
BO-PRO-1	DNA probe, cell-impermeant	462 nm	481 nm	BO-PRO™-3	DNA probe, cell-impermeant	575 nm	599 nm
PO-PRO-1	DNA probe, cell-impermeant	435 nm	455 nm	PO-PRO™-3 YOYO®-1 YOYO-3 YOYO-1 YOYO-3 YOYO-1 YOYO-3	DNA probe, cell-impermeant " " " " " "	539 nm 491 nm 612 nm 491 nm 612 nm 514 nm 642 nm	567 nm 509 nm 631 nm 509 nm 631 nm 533 nm 660 nm

### References

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#### BD Biosciences

Two Oak Park  
Bedford, MA 01730 USA  
tel: 877.232.8995  
fax: 800.325.9637

#### BD

2280 Argentinia Road  
Mississauga, Ontario  
Canada L5N 6H8  
tel: 866.979.9408  
fax: 800.565.0897

#### BD

Akasaka Garden City,  
Akasaka 4-15-1, Minato-ku,  
Tokyo, 107-0052 Japan  
tel: (81) 24 593 5405  
fax: (81) 24 593 5761

#### BD Biosciences

Singapore Branch  
30 Tuas Avenue 2  
Singapore 639461  
tel: (65) 6861 0633  
fax: (65) 6860 1590

#### BD Biosciences

Erembodegem-Dorp 86  
9320 Erembodegem, Belgium  
tel: (32) 53 720 211  
fax: (32) 53 720 450  
contact\_bdb@europe.bd.com

#### BD Biosciences

4 Research Park Drive  
Macquarie University Research Park  
North Ryde NSW 2113 Australia  
tel: (800) 656 100  
fax: (612) 8875 7200  
aus\_customerservice@bd.com

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