

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* ¹
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 ¹⁻³
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 ³
(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 ⁴

*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™ DiI₁₂(3) Fluorescent Dye (Cat. No. 354218), visit our website at: 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 cm² 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.³ 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.¹ **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 Ca²⁺/Mg²⁺ 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

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 ²⁺ probe, ratiometric	300-400 nm	510 nm	Fura Red™	Ca ²⁺ 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
Fluoroblok™							
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 ²⁺ probe, fixed λ	322 nm	435 nm	BD™ Calcein AM (Cat. No. 354216 and 354217) Calcium Orange™ AM Calcium Crimson™ AM	Ca ²⁺ probe, fixed λ " "	494 nm 549 nm 589 nm	517 nm 576 nm 615 nm
Fluoroblok™ DNA-blockers							
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
Fluoroblok™ DNA-bind derivatives							
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-FR O-1	DNA probe, cell-impermeant	462 nm	481 nm	BO-FR O™-3	DNA probe, cell-impermeant	575 nm	599 nm
PO-FR O-1	DNA probe, cell-impermeant	435 nm	455 nm	PO-FR O™-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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