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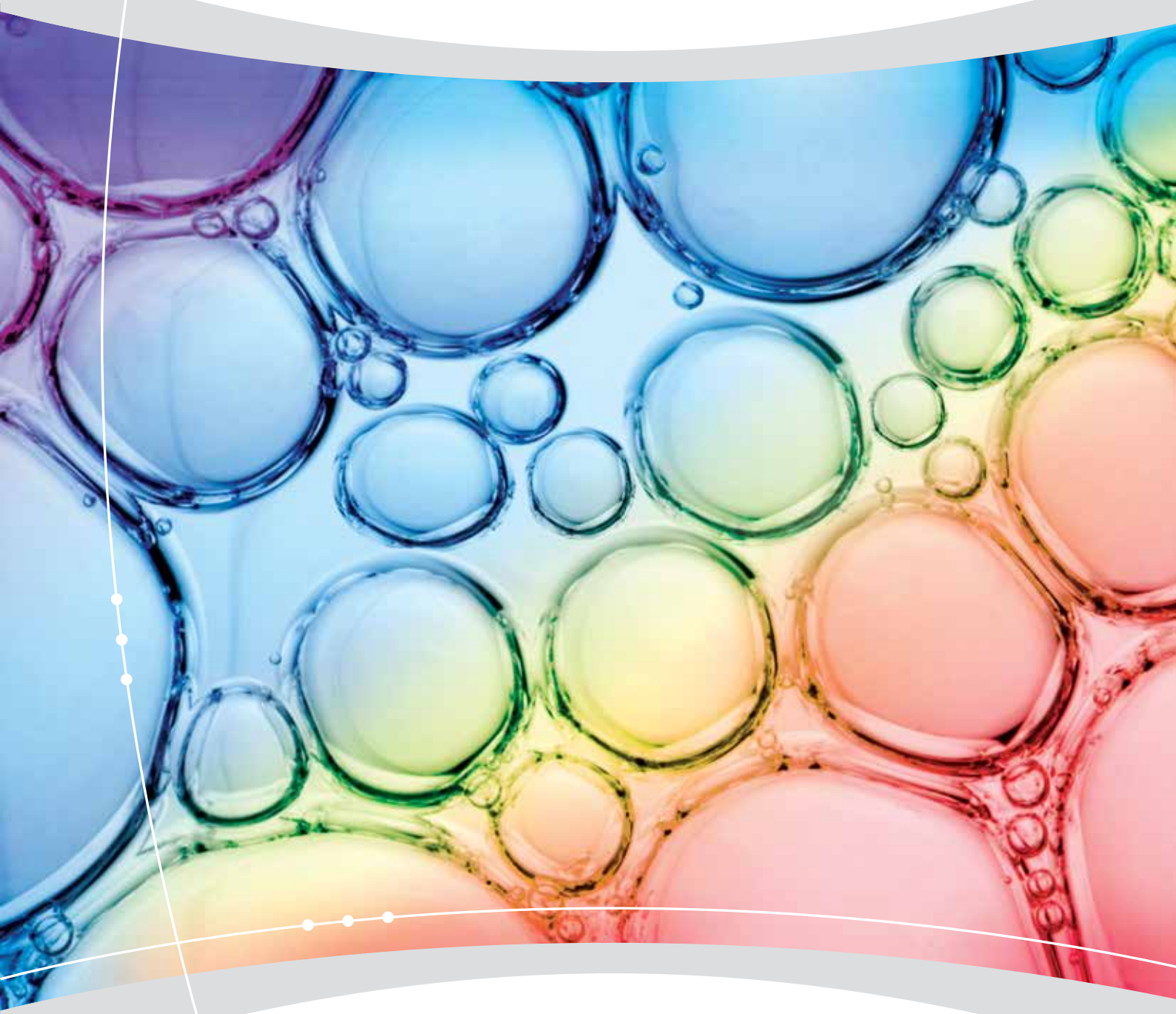
eBioscience

Beads, Buffers and Dyes

Support Systems for Flow Cytometry



eBioscience, an Affymetrix company, is committed to developing and manufacturing high-quality, innovative reagents using an ISO certified process. As a provider of more than 11,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in the areas of innovation, quality and value.

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Overview: Beads, Buffers and Dyes

Is your data affected by lot-to-lot variations and unreliable product quality? Ensuring experimental conditions are consistent and data is reproducible can be challenging. In response to your concerns, eBioscience has developed high-quality products that are validated and optimized in our own buffers and reagents, ensuring reproducible results.

eBioscience is an industry leader and manufacturer of organic dyes and fluorochrome-conjugated antibodies to both novel and established clones. eFluor® Organic Dyes are a proprietary line of fluorophores designed by eBioscience for superior optical performance and detection in applications using laser-based systems, notably flow cytometry.

Many support products have been optimized and simplified by our research and development team, who are experts in flow cytometry, to reduce error, thereby saving your laboratory time and money.

Finding the correct products for your research is easy

All eFluor fluorophores are named according to their emission wavelength and are fully compatible with protein based dyes, such as PE and APC, other organic fluorophores and semi-conductor quantum dots. These features, combined with a broad biological portfolio, allow for easy dye selection when optimizing multicolor panel design for flow cytometry.

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Cell Preparation

Simplify steps

Preparing cells for experimental procedure can be time consuming and laborious. Using multi-purpose or multi-function reagents saves time and reduces the need for multiple products. eBioscience provides buffers with the ability to fix/lyse and store cells in one step, while red blood cell lysis buffers are suitable for multiple species.

Red blood cell lysis

Removal of red blood cells (RBC) is necessary prior to using cell suspensions for flow cytometry and functional assays. eBioscience provides lysis buffers containing ammonium chloride, which are suitable for use before or after staining with antibodies in multiple species.

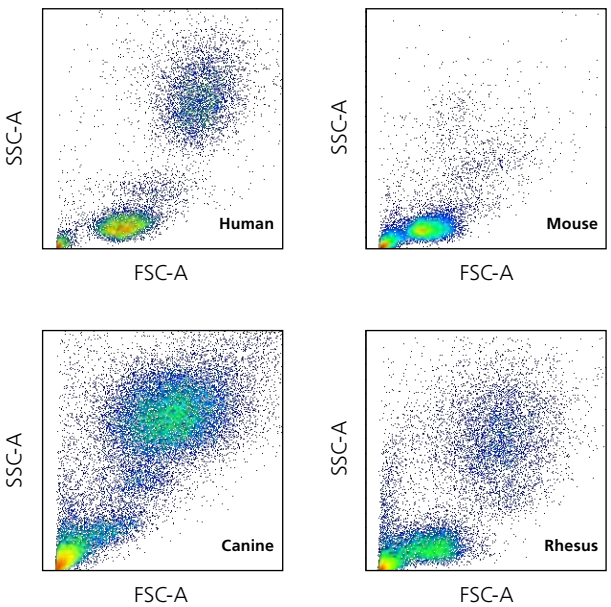
10X RBC Multi-Species Lysis Buffer

This amonium chloride buffer lyses red blood cells with minimal effect on leukocytes, and is suitable for use with **canine, human, mouse, rat and non-human primate** samples, such as peripheral blood and spleen.

1X RBC Lysis Buffer

The 1X Red Blood Cell Lysis Buffer is formulated for convenience, providing optimal erythrocyte lysis in single-cell suspensions of mouse hematopoietic tissues, such as spleen and mouse peripheral blood. The buffer lyses red cells with minimal effect on leukocytes when used as instructed. However, nucleated red cells are not effectively lysed with ammonium chloride. RBC lysis is not necessary when working with mouse thymus and lymph nodes.

One buffer–multiple species



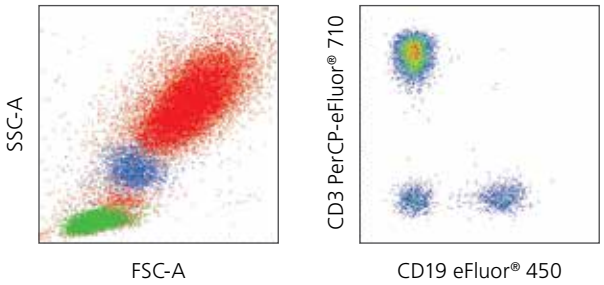
Lysis of normal peripheral blood in multiple species using 10X RBC Lysis Buffer (cat. no. 00-4300).

1-step Fix/Lyse Buffer

- Simple to use
- Suitable for tandem dyes
- Store fixed and stained cells

This solution fixes and lyses RBCs in one step while maintaining the fluorochrome antibody stained population. It is suitable for lysis of red blood cells after staining peripheral blood cells with fluorochrome-conjugated antibodies. There is minimal change in FRET efficiency of stained leukocytes when using tandem dye conjugated antibodies. 1-Step Fix/Lyse Buffer can be used as a storage buffer with little to no effect on fluorochromes, particularly tandem dyes.

Fix and lyse in one step



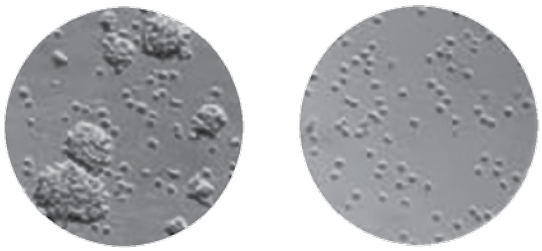
Normal human peripheral blood cells were stained with Anti-Human CD45 FITC (cat. no. 11-9459), Anti-Human CD3 PerCP-eFluor® 710 (cat. no. 46-0037) and Anti-Human CD19 eFluor® 450 (cat. no. 48-0199) and then incubated with eBioscience, freshly diluted 1-step Fix/Lyse Solution for 15 minutes at room temperature. Cells were spun, washed once in flow stain buffer, then analyzed (left). CD45+ granulocytes (red), monocytes (blue) and lymphocytes (green) can be seen in the forward vs. side scatter plot of total viable cells (right). Analysis of CD45+ lymphocytes.

Lysis Buffers At-a-Glance			
Cat. No.	**Name**	**Target**	**Feature**
00-4300	10X RBC Lysis Buffer (Multi-species)	▪ Red blood cells ▪ Peripheral blood and hematopoietic tissues, such as spleen	▪ Optimized for multiple species: Human, mouse, rat & non-human primate ▪ Use before or after staining ▪ Excellent choice for whole blood
00-4333	1X RBC Lysis Buffer	▪ Red blood cells ▪ Mouse hematopoietic tissues, such as spleen and human peripheral blood	▪ Suitable for mouse and human tissues
00-5333	1-step Fix/Lyse Solution (10X)	▪ Human whole blood ▪ Lyses non-nucleated erythrocytes	▪ Use in human whole blood ▪ Lyse & Fix cells in 10 minutes ▪ Allows storage of cells ▪ Eliminates gradient centrifugation ▪ Compatible with organic dyes, semi-conductor quantum dots and tandem dyes

Dissociation and detachment

Accumax® – Cell aggregate dissociation medium

Effectively dissociates cell clumps in a variety of cell lines, including hybridomas, CHO, BHK, 293, COS and Sf9 cells for cell counting, viral transfection assays, cell sorting, flow cytometry and bioreactor scale-up.



Accumax cell dissociation. Before, cell aggregates; 100x magnification. (left) After, same cells, following Accumax treatment; 100x magnification. (right)

Accutase® – Enzyme cell detachment medium

- Excellent for stem cells
- Greater cell recovery
- No neutralization

Harmlessly detaches cells from cultureware, allowing for the analysis of cell surface markers; virus growth assays; quiescence assays by serum starvation; transformation assays by oncogene transfection; neural crest cell migration assays; cell proliferation; cell haptotaxis; tumor cell migration assays; routine cell passage; production scale-up (bioreactor); and flow cytometry. Accutase is effective on: fibroblasts, keratinocytes, vascular endothelial cells, hepatocytes, vascular smooth muscle cells, hepatocyte progenitors, primary chick embryo neuronal cells, bone marrow stem cells, adherent CHO and BHK cells, macrophages, 293 cells, L929 cells, immortalized mouse testicular germ cells, 3T3, Vero, COS, HeLa, NT2, MG63, M24 and A375 metastatic melanoma, gliomas U251 and D54, HT1080 fibrosarcoma cells and Sf9 insect cells.

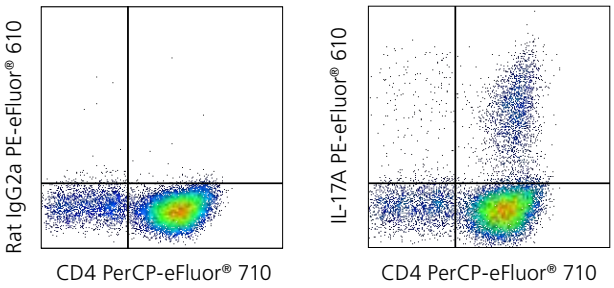
Cell Dissociation & Detachment		
Description	Application	Cat. No.
Accumax - Cell Aggregate Dissociation Medium	FA	00-4666
Accutase - Enzyme Cell Detachment Medium	FA	00-4555

Cell Treatments

Cell stimulation and protein accumulation

Cell type and experimental procedure are important factors to consider when stimulating cells. Stimulants can induce a variety of surface proteins, transcription factors, chemokines, cytokines and growth factors. One commonly used stimulant is a combination of PMA (Phorbol ester activates protein kinase C) and Ionomycin (calcium ionophore). eBioscience offers a Cell Stimulation Cocktail (cat. no. 00-4970), which includes both stimulants. However, for more specific stimulation of T-lymphocytes, CD3 and CD28 antibodies are a great option. Additionally, lipopolysaccharide (LPS) or CpG are used to induce IL-6, IL-10 or TNFα production by monocytes, macrophages and dendritic cells. Cytokines, chemokines and other secreted proteins are generally detected at low levels in resting cells, therefore they require some sort of stimulation to induce expression for flow cytometric detection. Once the proteins are expressed, it is necessary to block secretion, preventing movement through the secretory pathway at the endoplasmic reticulum with Brefeldin A, or at the Golgi apparatus with Monensin, which allows accumulation to reach detectable levels.

Stimulate cells with a cocktail



Splenocytes were cultured under Th17-polarizing conditions for 6 days then restimulated for 5 hours with the Cell Stimulation Cocktail (plus protein transport inhibitors) (cat. no. 00-4975). Cells were intracellularly stained with Anti-Mouse CD4 PerCP-eFluor® 710 (cat. no. 46-0042) and Rat IgG2a K Isotype Control PE-eFluor® 610 (cat. no. 61-4321) (left) or Anti-Mouse/ Rat IL-17A PE-eFluor® 610 (cat. no. 61-7177) (right) using the Intracellular Fixation & Permeabilization Buffer Set (cat. no. 88-8824) and protocol. Viable cells, as determined by Fixable Viability Dye eFluor® 660 (cat. no. 65-0864), in the lymphocyte gate were used for analysis.

Cell Stimulation & Inhibition		
Description	**Application**	**Cat. No.**
Cell Stimulation Cocktail (500X)	FC, ELISA, FA	00-4970
Cell Stimulation Cocktail (plus protein transport inhibitors) (500X)	FC, FA	00-4975
Protein Transport Inhibitor Cocktail (500X)	FC, FA	00-4980
Brefeldin A Solution (1000X)	FC	00-4506
Monensin Solution (1000X)	FC	00-4505

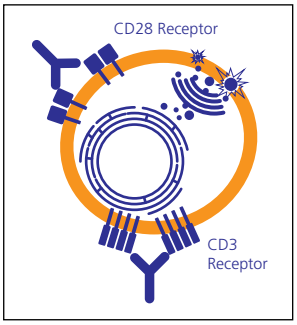
Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Cytokine Activation, Re-Stimulation & Blocking At-a-Glance			
Mouse Cytokine	**Activation**	**Re-Stimulation**	**Intracellular Block**
GM-CSF	ConA /IL-2	Anti-CD3 + Anti-CD28	Brefeldin A
IFNγ	ConA /IL-2	Anti-CD3 + Anti-CD28	Brefeldin A
IL-1α	mIFNγ /LPS	-	Brefeldin A
IL-2	ConA	Anti-CD3 + Anti-CD28	Brefeldin A
IL-4	Th2 polarized	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
IL-5	ConA	Anti-CD28	Brefeldin A
IL-10	ConA	Anti-CD3 + Anti-CD28	Brefeldin A
IL-12/IL-23 (p40)	LPS	-	Brefeldin A
IL-13	Th2 polarized	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
IL-22	Th17 polarized	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
MCP-1/ CCL2	LPS	-	Brefeldin A
TNFα	ConA	Anti-CD3 + Anti-CD28	Brefeldin A
IL-1β	LPS	-	Monensin
IL-6	LPS	-	Monensin
IL-17A	Th17 polarized	PMA + Ionomycin or Cell Stimulation Cocktail	Monensin
IL-17F	Th17 polarized	PMA + Ionomycin or Cell Stimulation Cocktail	Monensin
IL-21	Th17 polarized	PMA + Ionomycin or Cell Stimulation Cocktail	Monensin
IL-23 p19	mGM-CSF	LPS	Monensin
Human Cytokine	**Activation**	**Re-Stimulation**	**Intracellular Block**
IFNγ	PMA /Ionomycin	-	Brefeldin A
IL-1β	LPS	-	Brefeldin A
IL-1RA	LPS	-	Brefeldin A
IL-2	PMA /Ionomycin	-	Brefeldin A
IL-4	PMA /Ionomycin	-	Brefeldin A
IL-5	Th2 polarizing cultures	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
IL-6	LPS	-	Brefeldin A
IL-12/ IL-23 (p40)	hIFNγ/LPS	-	Brefeldin A
IL-13	Anti-CD3 + Anti-CD28	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
IL-17A	Th17 polarizing cultures	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
IL-21	PMA /Ionomycin	-	Brefeldin A
IL-22	Th17 polarizing cultures	PMA + Ionomycin or Cell Stimulation Cocktail	Brefeldin A
TNFα	PMA /Ionomycin	-	Brefeldin A
G-CSF	LPS	-	Monensin
GM-CSF	PMA /Ionomycin	-	Monensin
IL-1α	LPS	-	Monensin
IL-9	Th2 polarizing cultures	PMA + Ionomycin or Cell Stimulation Cocktail	Monensin
IL-10	Th2 polarizing cultures	PMA + Ionomycin or Cell Stimulation Cocktail	Monensin
IL-23 p19	hGM-CSF+hIL-4	LPS	Monensin
MCP-1/ CCL2	LPS	-	Monensin
RANTES/ CCL5	LPS	-	Monensin
TNFβ	Th1 polarizing cultures	PMA + Ionomycin or Cell Stimulation Cocktail	Monensin

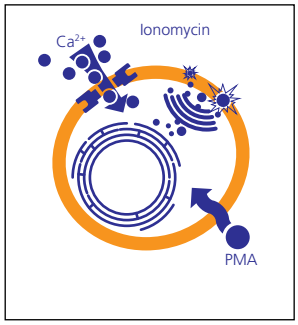
Annotations: mouse PEC=mouse thioglycolate-elicited peritoneal macrophages; ConA=Concanavalin A; Iono=Ionomycin; LPS=Lipopolysaccharide; PMA=Phorbol Myristate Acetate

Visit **ebioscience.com/knowledge-center.htm** for more information and a complete listing of products

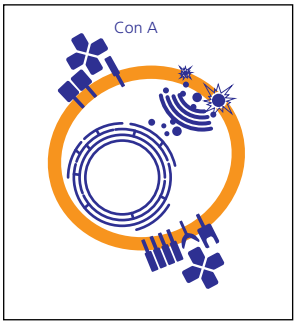
Activation and stimulation options



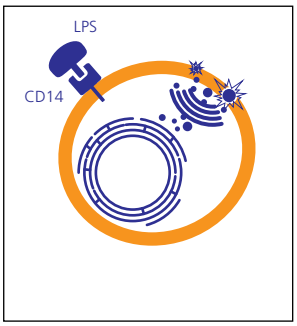
CD28/CD3
TCR-mediated activation and co-stimulation



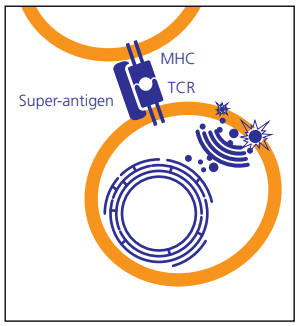
Ionomycin and PMA
Leukocyte activation and stimulation



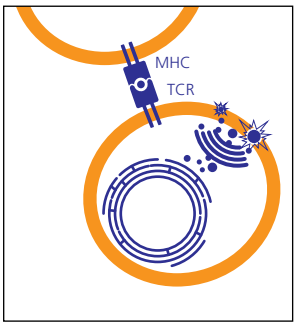
Con A
Lectin-mediated Mitogenic activation



LPS
Leukocyte activation



TCR and MHC receptor
T cell activation



TCR-MHC/peptide
T cell activation

T cells require two stimulus for activation with the first signal, which is antigen specific, generated through the T cell receptor (TCR) interacting with the major histocompatibility complex (MHC) on antigen presenting cells (APC). The second interaction occurs between molecules on the APC and T cells. B cells bind antigens with the B cell receptor (BCR), which are then presented on the MHC II molecules, resulting in activation of B cell binding to TCR and the MHC complex.

3

Blocking

Block Fc-mediated binding

- Reduce background
- Improve data

High background reduces data quality, making analysis difficult, but by using human Fc receptor blocking reagents or Anti-Mouse CD16/32, optimal staining and signal-to-noise ratios can be achieved.

Fc-blocking reagents are used to inhibit non-specific Fc Receptor (FcγR)-mediated binding of antibodies, thereby allowing optimal staining and signal-to-noise ratio during flow cytometric analysis. Four different classes of FcγR are expressed at varying levels in multiple cell lineages, with high expression observed in myeloid and B cells. The biological function of the FcγR, including initiation of endocytosis, phagocytosis and antigen presentation, is elicited upon binding of host-immunoglobulin. Binding of FcγR to monoclonal antibodies varies depending upon isotype.

Human & Mouse Background Blocking At-a-Glance

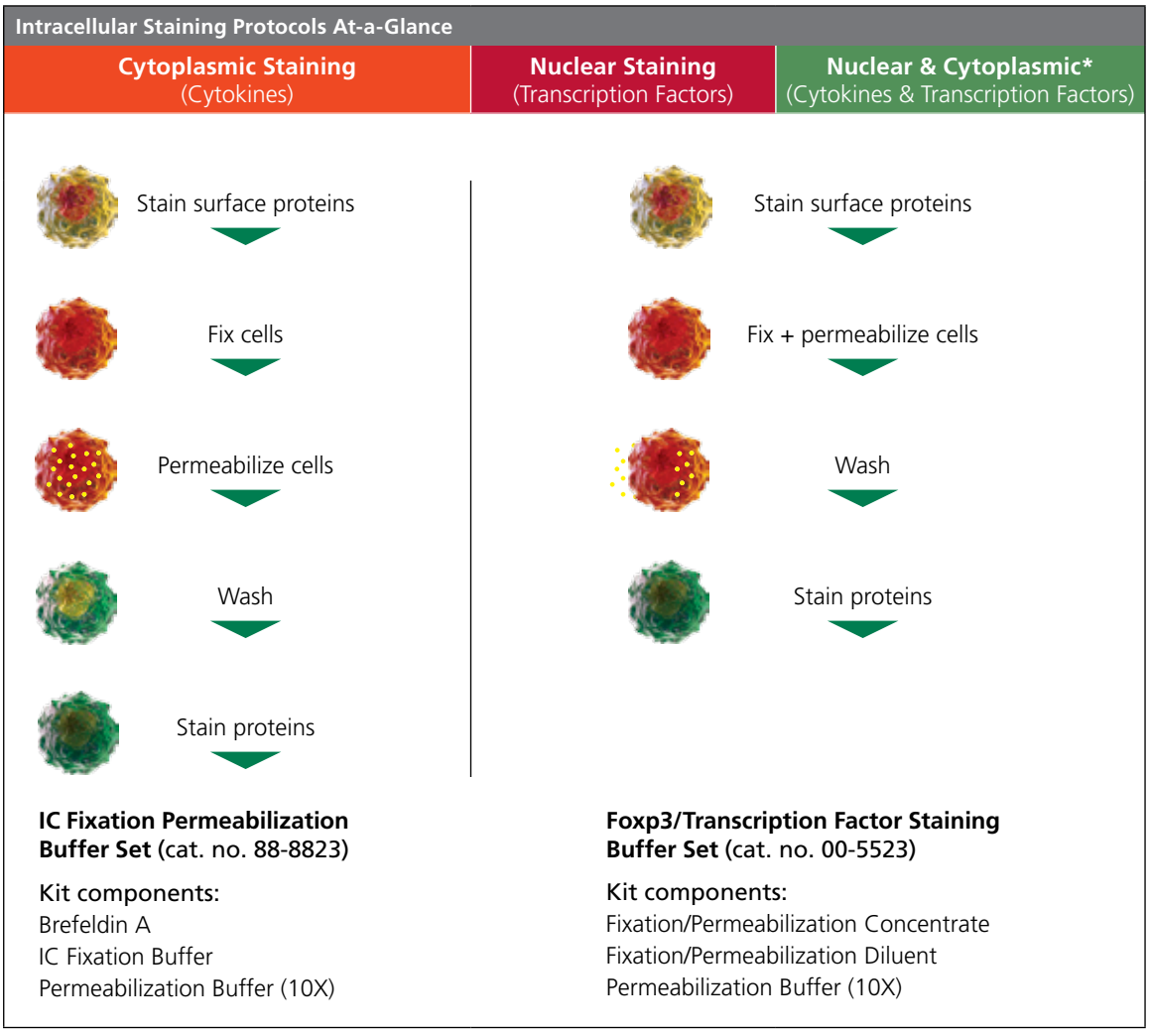
Name	Cat. No.	Target	Feature
Human Fc Receptor Binding Inhibitor Functional Grade Purified	16-9161	▪ Four different Fc γ receptor classes: FcγRI (CD64) FcγRII (CD32) FcγRIII (CD16) FcγRIV ▪ Expressed at varying cell lineages: myeloid granulocyte B cells	▪ Inhibits non-specific Fcγ receptor mediated binding of antibodies to cell surface Fc receptors ▪ Does not contain sodium azide
Human Fc Receptor Binding Inhibitor Purified	14-9161		▪ Contains sodium azide
Anti-Mouse CD16/CD32 Functional Grade Purified	16-0161	▪ Low affinity receptors for mouse IgG Fc: CD16 (FcγIII Receptor) CD32 (FcγII Receptor) ▪ Expressed by: B cells monocyte/macrophages NK cells neutrophils	▪ Does not contain sodium azide
Anti-Mouse CD16/CD32 Purified	14-0161		▪ Contains sodium azide

Surface and Intracellular Staining

Choosing the best buffer system

Cell signaling is critical for cell growth, proliferation and repair. Understanding signal transduction pathways can provide important information on disease development and progression. Analysis of proteins involved in signaling pathways by flow cytometry requires consideration as to the location of target proteins. Once known, the appropriate buffer for surface and intracellular staining, whether it is cytoplasmic or nuclear, can be chosen. eBioscience buffers have been optimized for nuclear proteins, such as transcription factors, in addition to cytoplasmic and secreted proteins.

When combining staining of proteins that localize to different regions, choosing the correct buffer becomes more challenging. For example, in order to obtain optimal staining of a transcription factor, it is recommended to use the Foxp3/Transcription Factor Staining Buffer Set, however when including cytokine staining, a dilemma may occur. The following chart provides a general rule. There may be a decrease in brightness with one buffer system over another, therefore each antibody should be optimized independently to validate the staining pattern.



Legend: IC = Intracellular, Perm = Permeabilization

Intracellular cytoplasmic staining

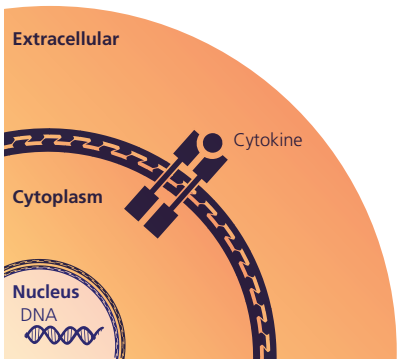
Cytoplasmic staining requires fixation to crosslink protein, thereby preventing cell contents from escaping once it has been permeabilized. Intracellular fixation and permeabilization buffers are ideal for optimal detection of cytoplasmic proteins, cytokines and other secreted proteins. The eBioscience Intracellular Fixation & Permeabilization Buffer Set is designed for use when staining proteins, such as adaptor proteins β-catenin, actin and tubulin, in addition to receptor proteins in which the antibody recognizes a cytoplasmic version (CD152 (CTLA-4)) or cytoplasmic domain.

Secreted protein: cytokine, chemokine and growth factors

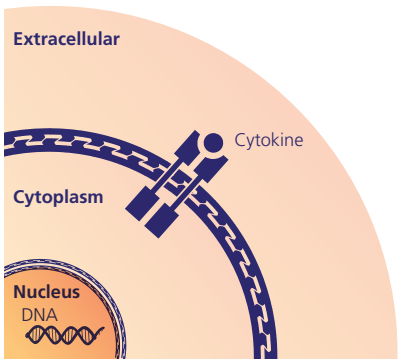
Proteins that go through the secretory pathway, such as cytokines, chemokines and growth factors also require fixation and permeabilization in addition to protein accumulation for optimal detection in flow cytometry analysis. This can be accomplished by blocking protein movement to different organelles and secretion through the use of transport inhibitors.

Cytoplasmic and Cytokine Buffers At-a-Glance

Name	Cat. No.	Target	Feature
Intracellular Fixation & Permeabilization Buffer plus Brefeldin A	88-8823	▪ Cytokines ▪ Cytoplasmic proteins	▪ Contains Brefeldin A ▪ Contains cat. no. 00-8222 and 00-8333
Intracellular Fixation & Permeabilization Buffer Set	88-8824		▪ Contains cat. no. 00-8222 and 00-8333
Intracellular Fixation Buffer	00-8222		▪ Increases signal-to-noise ratios
Permeabilization Buffer (10x)	00-8333		▪ Permeabilizes cells prior to intracellular staining



Intracellular Staining



Nuclear Staining

Nuclear staining

Transcription factors are key groups of molecules involved in regulating gene expression by modulating the synthesis of messenger RNA. Cellular functions, such as cell proliferation, differentiation and apoptosis, are mediated by transcription factors that are either up-regulated or blocked, causing inflammatory responses and tumorigenesis. Foxp3 is considered the master regulator of T regulatory cells (Treg). Induction of the Foxp3 gene in normal naïve T cells converts them to Treg-like cells with *in vivo* and *in vitro* suppressive function, indicating that Foxp3 plays a key role in controlling expression of critical suppression-mediating molecules. Elucidation of the molecular targets of Foxp3 will be fundamental to a complete understanding of the suppressive functions of Tregs. eBioscience, having mapped the epitopes of Foxp3 antibodies, is an industry leader in providing tools for the identification of Tregs. The Foxp3/Transcription Factor Staining Buffer Set, although originally developed for Foxp3 staining, has been optimized for use with nuclear factors, cytosolic proteins, secreted proteins and transcription factors in addition to cytokines. These include Eomes, T-bet, Gata-3, IRF4, phospho-H2Ax, Rorγ(t), Egr2, Ki-67 and Sox2. This buffer can also be used to stain many secreted proteins (IL-17A and Granzyme B).

Nuclear Staining Buffers At-a-Glance

Description	Cat. No.	Target	Feature
Foxp3/Transcription Factor Staining Buffer Set	00-5523	▪ Nuclear factors ▪ Transcription factors ▪ Cytosolic proteins ▪ Secreted proteins	▪ Works with all transcription factors and most nuclear proteins ▪ Contains cat. no. 00-5123, 00-5223 & 00-8333
Fixation/Permeabilization Concentrate	00-5123	▪ Transcription factors ▪ Cytokines	▪ Works with all transcription factors and most nuclear proteins
Fixation/Permeabilization Diluent	00-5223		
Foxp3 Fixation/Permeabilization Concentrate and Diluent	00-5521	▪ Nuclear factors ▪ Transcription factors ▪ Cytosolic proteins ▪ Secreted proteins	▪ Works with all transcription factors and most nuclear proteins ▪ Contains cat. no. 00-5123 & 00-5223

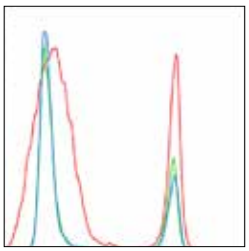
Staining Buffer At-a-Glance

Description	Cat. No.	Target	Feature
Flow Cytometry Staining Buffer	00-4222	▪ Tissue culture cell preparation ▪ Lymphoid tissue cell preparation ▪ Non-lymphoid tissue cell preparation ▪ Isolation of PBMC from whole blood	▪ Optimized for flow cytometry ▪ Ideal pH

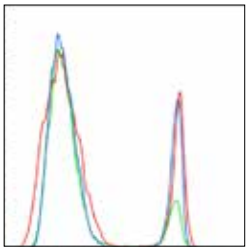
Fixation buffers

Fixation buffers for flow cytometry are generally used after surface staining and as an early step during intracellular staining protocols. However, fixation buffers are also a great reagent on occasions when you cannot gain access to the cytometer for hours or days but need to preserve both the cells and staining. Intracellular Fixation Buffer (cat. no. 00-8222) and 1-step Fix/Lyse Buffer (cat. no. 00-5333) for flow cytometry are manufactured with the highest quality materials, allowing for safe cell storage when it is impossible to run samples immediately. eBioscience fixatives have minimal impact on tandem dye brightness or compensation values when compared to freshly stained live cells.

eBioscience fixatives have minimal impact on tandem brightness or compensation



PE-Cy7



APC-eFluor® 780

IC Fixation Buffer for 3 days
(100 µL cells+ 100 µL IC Fix Buffer) Compensation: 5%

1-Step Fix/Lyse for 3 days
(100 µL cells + 2 mL Fix/Lyse Buffer) Compensation: 5%

Live cells
Compensation: 4.5%

Tandem dyes can be stored in eBioscience fixatives with virtually no change in compensation/FRET efficiency when compared to freshly stained and analyzed cells. The data shows minimal impact to tandem brightness and compensation of mouse CD4 (RM4-5) after being stored for periods of three days in 1-step Fix/Lyse or solution.

Simple Storage Protocol	
 Stain	1. Finish standard staining protocol.
 Wash	2. Wash and decant cells in 100 µL staining buffer.
 Fix & Store	3. Add eBioscience Fixative: 100 µL of IC Fixation Buffer or 2 mL 1-step Fix/Lyse Solution. 4. Store at 2-8°C, in the dark, for up to 3 days.
 Wash and Analyze	5. Wash and analyze using standard protocol when ready.

Cellular Activity

Cell condition

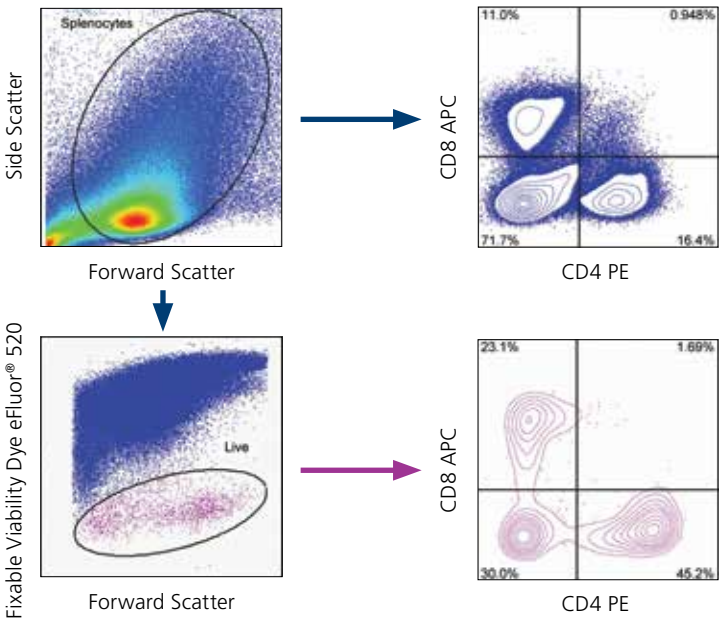
Excluding dead cells from data is recommended for all staining protocols to ensure accurate data. Dead cells can be "sticky", resulting in non-specific binding, which causes high background staining and false positives. Gating based on forward and side scatter (FSC/SSC) is not a reliable method for distinguishing dead cells. Viability dyes ensure dead cells are removed from analysis, thereby reducing non-specific binding and background in addition to better peak separation. eBioscience offers several options for ensuring only live cells are analyzed, such as DNA-intercalating dyes, enzymatically sensitive dyes and fixable viability dyes.

eFluor® Fixable Viability Dyes

- Ready-to-use format
- Excludes dead cells easily
- Improves data quality

eBioscience eFluor® Fixable Viability Dyes (FVD) penetrate compromised membranes, irreversibly labeling dead cells from all species prior to fixation and permeabilization. Dead cell populations have high fluorescent intensity, allowing for easy exclusion, thereby improving data quality. Fixable Viability Dyes eFluor® 455UV, eFluor® 450, eFluor® 506, eFluor® 520, eFluor® 660 and eFluor® 780 are permanent dyes suitable for UV, violet, blue and red lasers that will not wash out of cells. Unlike 7-AAD and propidium iodide, cells labeled with Fixable Viability Dyes can be cryopreserved or fixed, permeabilized and stained for intracellular antigens without losing staining intensity of the dead cells.

The importance of viability dyes



Mouse splenocytes were stimulated with ConA. for 4 days

Top row: Total cells analyzed for CD4 and CD8, based on a forward and side scatter gate.

Bottom row: Total cells were subgated on viable cells, based on FVD eFluor 520, then analyzed for CD4 and CD8.

7-AAD Viability Dye and Propidium Iodide (PI) Staining Solution

- Ready-to-use format
- Dead cell discrimination

7-AAD Viability Dye and Propidium Iodide (PI) Staining Solution mark non-viable cells by intercalating with the DNA of dead cells in a concentration-dependent manner. The nucleic acid of viable cells will not be accessible to the dye, thereby preventing staining. They are not recommended for viability gating if the cells will be fixed. 7-AAD and PI may also be used for flow cytometric analysis of the cell cycle. PI binds to the double stranded DNA of dead cells, but is excluded from cells with intact plasma membranes.

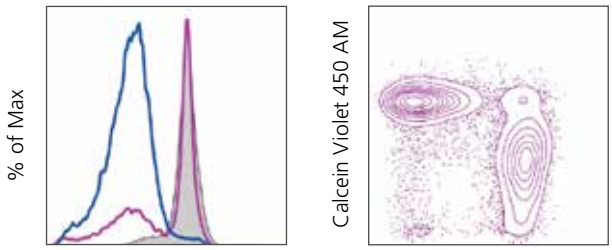
Dead Cell Staining At-a-Glance							
Description	Format	Utility	Viability	Application	Excitation	Emission	Cat. No.
UV Laser							
Fixable Viability Dye eFluor® 455UV	Solution/ Ready-to-use	Irreversibly labels dead cells prior to cryopreservation, fixation and/or permeabilization procedures. Cells can be washed without loss of staining on dead cells, allowing for exclusion during analysis	Dead Cells	FC	350 nm	455 nm	65-0868
Violet Laser							
Fixable Viability Dye eFluor® 450	Solution/ Ready-to-use	Irreversibly labels dead cells prior to cryopreservation, fixation and/or permeabilization procedures. Cells can be washed without loss of staining on dead cells, allowing for exclusion during analysis	Dead Cells	FC	405 nm	450 nm	65-0863
Fixable Viability Dye eFluor® 506					405 nm	506 nm	65-0866
Blue Laser							
Fixable Viability Dye eFluor® 520	Solution/ Ready-to-use	Irreversibly labels dead cells prior to cryopreservation, fixation and/or permeabilization procedures. Cells can be washed without loss of staining on dead cells, allowing for exclusion during analysis	Dead cells	FC	488 nm	522 nm	65-0866
Propidium Iodide (PI)		Binds to double stranded nucleic acid by intercalating between basepairs. Excluded from cells with intact plasma membranes. Use FL3 for viability exclusion. Analyze in FL2 when used as a counterstain for FITC Annexin V.				617 nm	00-6990
7-AAD Viability Staining Solution		Use in place of PI (propidium iodide) or in combination with PE (phycoerythrin) and FITC (fluorescein isothiocyanate) conjugated antibodies in multi-color analysis. Minimal spectral overlap between these emissions.				670 nm	00-6993
Red Laser							
Fixable Viability Dye eFluor® 660	Solution/ Ready-to-use	Irreversibly labels dead cells prior to cryopreservation, fixation and/or permeabilization procedures. Cells can be washed without loss of staining on dead cells, allowing for exclusion during analysis	Dead Cells	FC	633 nm	660 nm	65-0864
Fixable Viability Dye eFluor® 780					633 nm	780 nm	65-0865
Multi-Laser Pack							
Fixable Viability Dye eFluor® 506/780	Solution/ Ready-to-use	Irreversibly labels dead cells prior to cryopreservation, fixation and/or permeabilization procedures. Cells can be washed without loss of staining on dead cells, allowing for exclusion during analysis	Dead Cells	FC	405/633 nm	506/780 nm	65-2860

Calcein AM, Calcein Blue AM, Calcein Violet 450 AM

Calcein labeling dyes cross the cell membrane easily, selectively labeling live cells for analysis by flow cytometry or fluorescent microscopy; however apoptotic and dead cells with compromised cell membranes do not retain Calcein.

Calcein dyes are nonfluorescent until they cross the cell membrane of viable cells and are enzymatically processed by intracellular esterases to their fluorescent, membrane non-permeable form. Dead cells do not have intact cell membranes and cannot retain the cleaved Calcein dyes, nor do they have active esterases to cleave the Calceins to their fluorescent forms. Co-staining with Annexin V or 7-AAD is recommended to allow the greatest resolution between live and dead/apoptotic cells.

Calcein Violet 450 AM excited by the violet laser



Balb/c thymocytes were stained with 1 µM Calcein Violet 450 AM (cat. no. 65-0854) for 30 minutes at room temperature (left). Thymocytes were kept on ice overnight (shaded histogram) or cultured overnight at 37°C without (purple) or with (blue) 1 µM dexamethasone. Thymocytes cultured overnight without dexamethasone were also stained with Annexin V-APC (cat. no. 88-8007) allowing further discrimination between live and dead cells (right). Total cells were used for analysis.

Live Cell Labeling At-a-Glance							
Description	Format	Utility	Viability	Application	Excitation	Emission	Cat. No.
UV Laser							
Calcein Blue AM	Lyophilized	Membrane permeable live cell labeling dye Co-stain with Annexin V or 7-AAD for greatest resolution between live and dead/apoptotic cells	Live cells	FC/ Microscopy	360 nm	445 nm	65-0855
Violet Laser							
Calcein Violet 450 AM	Lyophilized	Membrane permeable live cell labeling dye Co-stain with Annexin V or 7-AAD for greatest resolution between live and dead/apoptotic cells	Live cells	FC/ Microscopy	408 nm	450 nm	65-0854
Blue Laser							
Calcein AM (Ultra Pure)	Lyophilized	Membrane permeable live cell labeling dye Co-stain with Annexin V or 7-AAD for greatest resolution between live and dead/apoptotic cells For improved resolution of live and dead/apoptotic cells using single color analysis, Calcein Blue AM or Calcein Violet 450 AM are recommended	Live cells	FC/ Microscopy	495 nm	515 nm	65-0853

Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Apoptosis

In early-stage apoptosis, the plasma membrane excludes viability dyes such as propidium iodide (PI), 7-AAD, or fixable viability dyes (FVD) such as FVD eFluor® 660 or eFluor® 780. These cells will stain with Annexin V due to phosphotidlyserine (PS) present in the inner plasma membrane moving to the outer membrane, but not a viability dye, distinguishing cells in early apoptosis. However, in late stage apoptosis, the cell membrane loses integrity, thereby allowing the DNA intercalating of FVD dyes to enter the interior of the cell. A viability dye can be used to resolve these late-stage apoptotic and necrotic cells (Annexin V, viability dye-positive) from the early-stage apoptotic cells (Annexin V positive, viability dye-negative).

Annexin V

Annexin V is a family of calcium-dependent phospholipid-binding proteins that preferentially binds to PS, thereby identifying apoptotic cells. Under normal physiologic conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet, marking cells as targets for phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin V in a calcium-dependent manner. eBioscience offers many fluorchrome-conjugated formats of Annexin V.

JC-1

JC-1 is a membrane permeable dye widely used for determining loss of mitochondrial membrane potential associated with apoptosis or cell stress in flow cytometry and fluorescent microscopy. The dye selectively enters the mitochondria where it reversibly changes color as membrane potentials increase (over values of about 80-100 mV). This property is due to the reversible formation of JC-1 aggregates upon membrane polarization which causes shifts in emitted light from 530 nm (i.e., emission of JC-1 monomeric form) to 590 nm (i.e., emission of J-aggregate) when excited at 488 nm. Both colors can be detected using filters for FITC and PE, respectively. JC-1 is both qualitative, with respect to shift from green to orange fluorescence emission, and quantitative, as measured by fluorescence intensity.

Detecting Cell Death		
Description	Application	Cat. No.
Annexin V Apoptosis Detection Kit eFluor® 450	FC	88-8006
Annexin V Apoptosis Detection Kit FITC	FC	88-8005
Annexin V-FITC Apoptosis Detection Kit	FC	BMS500FI
Annexin V-FITC Apoptosis Detection Kit	FC	BMS500FICE
Annexin V PE Apoptosis Detection Kit PE	FC	88-8102
Annexin V Apoptosis Detection Set PE-Cy7	FC	88-8103
Annexin V Apoptosis Detection Kit APC	FC	88-8007
Annexin V Apoptosis Detection Kit PerCP-eFluor® 710	FC	88-8008
Annexin V-Biotin Apoptosis Detection Kit	FC	BMS500BT
Binding Buffer for Annexin V	FC	BMS500BB
JC-1 Mitochondrial Membrane Potential Dye	FC, IHC	65-0851

Cell function

Calcium sensing reagents

Calcium Sensing Dye eFluor® 514

Membrane-permeable dyes, such as Calcium sensing dye eFluor® 514 can be used to monitor changes in intracellular free calcium concentrations in the cell using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Calcium Sensor Dye eFluor® 514 enters the cell with an acetoxymethyl (AM) ester group that is cleaved by cellular esterases yielding a membrane-impermeable dye fluorescing at ~520 nm when excited with the 488 nm laser. Calcium Sensor Dye eFluor® 514, like Fluo-3 and Fluo-4, is a commonly used dye among the visible light-excitable calcium indicators, but with increased cellular uptake and brightness, even at room temperature.

Indo-1 AM Calcium Sensor Dye

This membrane-permeable dye is used for determining changes in calcium concentrations within the cell using fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers. Once Indo-1 enters the cell, esterases cleave the AM ester group, yielding a membrane-impermeable dye with a peak excitation wavelength of 346 nm. Unbound Indo-1 has a peak emission at 485 nm. Upon binding calcium, the peak emission shifts down to 410 nm.

Flow cytometry enables Indo-1 AM to be measured over time and can be represented as a ratio of the two emission wavelengths. To optimize the ratio between the two emissions, unbound Indo-1 fluorescence should be collected using a filter above 485 nm (525 nm is a good option), while bound Indo-1 fluorescence should be collected using a filter below 400 nm. Because the emission profile of Indo-1 is broad, multicolor flow analysis using fluorochrome off the violet laser is not possible; however fluorochrome-conjugated antibodies utilizing the 488 nm or 633 nm laser lines are compatible with Indo-1.

Cell Monitoring		
Description	Application	Cat. No
Calcium Sensor Dye eFluor® 514	FC, IHC	65-0859
Indo-1 AM Calcium Sensor Dye	FC, IHC	65-0856
Indo-1 AM Calcium Sensor Dye (UltraPure Grade)	FC, IHC	65-0857

Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Cell tracking

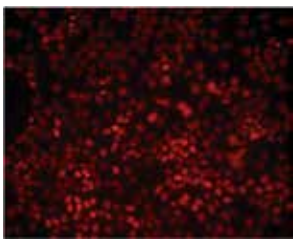
CellVue dyes offer a stable method to rapidly label the cell membrane of live cells with lipophilic dyes suitable for flow cytometry and microscopy.

Cell Tracking Dyes				
Description	Excitation	Emission	Application	Cat. No.
Violet Laser				
CellVue® Lavender Cell Labeling Kit	420 nm	461 nm	FC, ICC, IHC, FA	88-0873
Blue Laser				
CellVue® Jade Cell Labeling Kit	478 nm	508 nm	FC, ICC, IHC, FA	88-0876
Red Laser				
CellVue® Maroon Cell Labeling Kit	647 nm	667 nm	FC, ICC, IHC, FA	88-0870
CellVue® Plum Cell Labeling Kit	652 nm	671 nm	FC, ICC, IHC, FA	88-0871
CellVue® Burgundy Cell Labeling Kit	683 nm	707 nm	FC, ICC, IHC, FA	88-0872
CellVue® NIR780 Cell Labeling Kit	633 nm	776 nm	FC, ICC, IHC, FA	88-0875
CellVue® NIR815 Cell Labeling Kit	786 nm	814 nm	ICC, IHC, FA	88-0874
CellVue® Diluent C			FC, IHC	00-4501

CyTRAK Orange™ U2-OS human osteosarcoma cells, counterstained with CyTRAK Orange™ (courtesy of Biostatus).

CyTRAK Orange™

CyTRAK Orange™ is an anthraquinone dye with high affinity for double-stranded DNA. It is a membrane-permeable dye that can label live or fixed/dead cells. In flow cytometry, it can be used to distinguish nucleated and non-nucleated cells. In fluorescent microscopy, it can be used to identify and discriminate the nucleus and cytoplasm without the need for a second dye, due to its high intensity staining of the nucleus and low intensity staining of the cytoplasm. CyTRAK Orange is optimally excited from 488 to 550 nm with a peak emission of 610 nm.



DRAQ5™ Fixed and permeabilized MDCK cells stained with 10 nM DRAQ5™ nuclear stain (cat. no. 65-0880) (red), 20X.

DRAQ5™

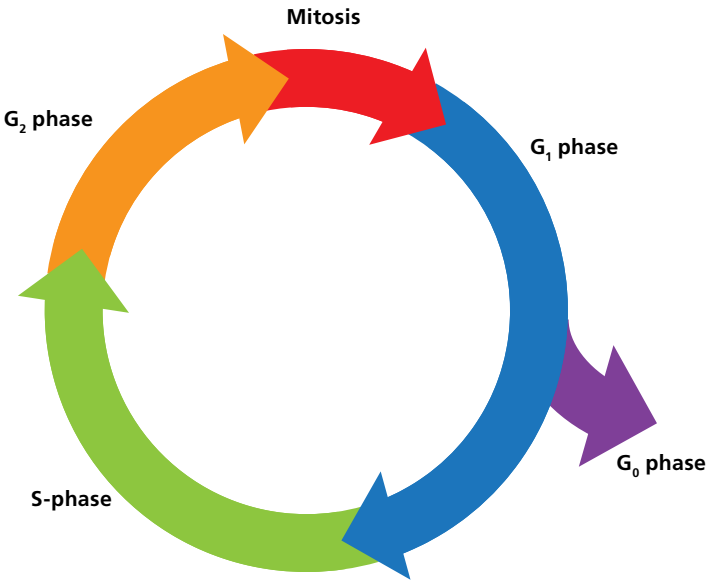
DRAQ5™ is an anthraquinone dye with high affinity for double-stranded DNA. It is a membrane-permeable dye that can label live or fixed/dead cells. In flow cytometry, this dye can be used to distinguish nucleated and non-nucleated cells. DRAQ5 can also be used to report nuclear DNA content for ploidy and cell cycle analysis because it binds DNA stoichiometrically. In fluorescent microscopy, it can be used as a nuclear counterstain. DRAQ5 can be excited from 488-647 nm with a peak emission of 670 nm.

Membrane Permeable Dyes		
Description	Application	Cat. No
CyTRAK Orange™	FC, ICC, IHC, FA	65-0881
DRAQ5™	FC, ICC, IHC, FA	65-0880

Application Key: BA = Bioassay; ELISA; ELISA (c) = ELISA capture; ELISA (d) = ELISA detection; ELISPOT (c) = ELISPOT capture; ELISPOT (d) = ELISPOT detection; FA = Functional Activity; FC = Flow Cytometry; FF = FlowCytomix™; IC Flow = Intracellular Staining/Flow Cytometry; ICC = Immunocytochemistry; IHC = Immunohistochemistry; IP = Immunoprecipitation; MIC = Microscopy; NU = Neutralizing; WB = Western Blot

Cell proliferation

The study of cell proliferation is important in the understanding of uncontrolled cell growth as a result of cancer, in addition to cell development, regulation and differentiation. Understanding the effects of gene addition or deletion and chemical additives on cells can be observed with proliferation assays.



Cell cycle analysis

G_0 phase: Resting cells have zero growth

G_1 phase: Enzyme synthesis is required for DNA replication

S-phase: DNA replication producing two identical sets of chromosomes

G_2 phase: Protein synthesis occurs

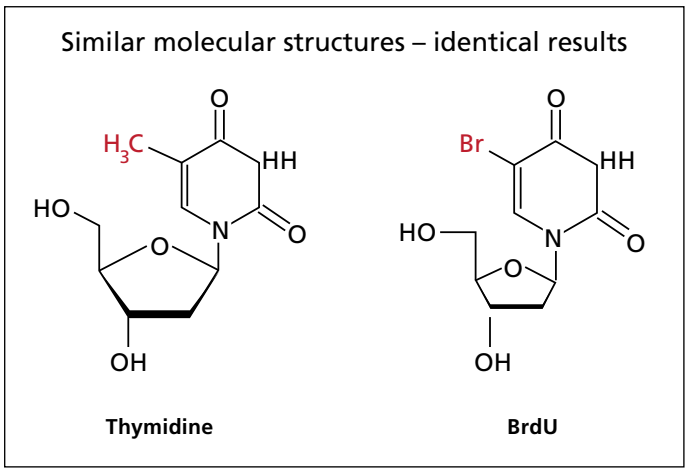
Mitosis: The nucleus and cell divide

Methods to Evaluate Cell Proliferation			
BrdU	**Ki-67**	**PCNA**	**Proliferation Dyes**
Measures cells in S-phase only	Measures proliferating cells at any cell cycle stage except G_0	Measures S-phase but also includes late G_1 phase	Measures generational proliferation
Pulse-labeling common to avoid cytotoxicity	BrdU is a subset of Ki-67 positive cells	Data supports IHC applications Not as robust for flow cytometry	Long-term labeling assay. Does not require fixation
In long-term culture, BrdU can be pulse-labeled and washed out Dividing cells will not incorporate BrdU so toxicity is diluted	Ki-67 and BrdU are used together in both IHC and flow cytometry		Cannot distinguish cell cycle phases of daughter cells

BrdU

- Fast Protocol
- 1-step proprietary buffer

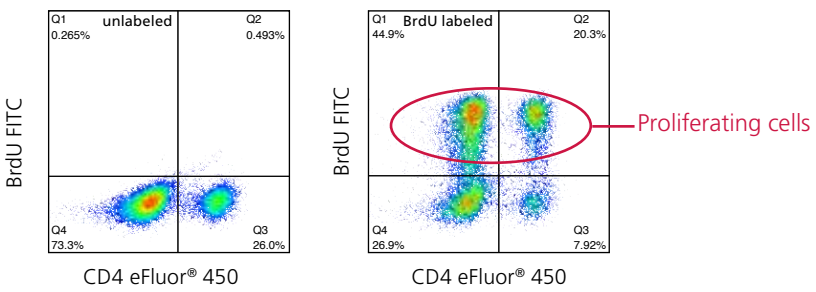
BrdU is a synthetic analog for thymidine, integrated into DNA during S-phase. BrdU incorporation is measured using an Anti-BrdU antibody showing at least one round of S-phase has been completed.



^{3}H- thymidine-/MTT assays are a sensitive and accurate way to measure overall proliferation, although information is unavailable as to which cells have gone through S-phase. A specific instrument is required for reading results, while BrdU is evaluated using a standard flow cytometer.

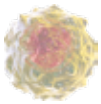
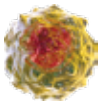
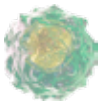
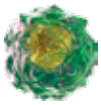
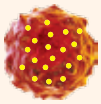
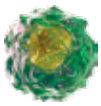
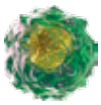
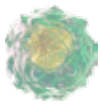
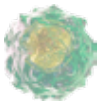
The eBioscience BrdU Proliferation Assay is simple and easy-to-use with few steps, faster results and a room temperature fix/perm protocol. The BrdU assay is compatible with staining both surface and intracellular targets. The BrdU Staining Buffer Set is optimized for use in the proliferation assay, enabling improved BrdU staining and consistency.

BrdU measures S-phase proliferation



Flow cytometry BrdU staining: Anti-Mouse CD3 and CD28 stimulated mouse splenocytes, either unlabled (left) or labeled with BrdU (right) were intracellularly stained with Anti-Mouse CD4 eFluor® 450 (cat. no. 48-0041) and Anti-BrdU FITC using the BrdU staining kit.

Comparative Workflow for BrdU Staining

Competitor Protocol	eBioscience Protocol
 1. Label cells with BrdU	 1. Label cells with BrdU
 2. Surface stain (optional)	 2. Surface stain (optional)
 3. Fix/Perm for **30 minutes** at room temperature	 3. Fix/Perm in BrdU Staining Buffer for **15 minutes** at room temperature*
 4. Incubate with perm buffer for **10 minutes** on **ice**	 4. Treat cells with DNase I for 1 hour at 37°C
 5. Fix cells for **5 minutes** at room temperature	 5. Stain for BrdU (and other cell surface/ intracellular antigens) for 20-30 minutes at 2-8°C
 6. Treat cells with DNase I for 1 hour at 37°C	6. Analyze samples
 7. Stain for BrdU (and other intracellular antigens) for 20-30 minutes at 2-8°C	
8. Analyze samples	

* Stopping point (up to 16 hours tested)

BrdU Proliferation Staining Kits	
Description	**Cat. No.**
eFluor® 450	8848-6600-42
FITC	8811-6600-42
PE	8812-6600-42
PerCP-eFluor® 710	8846-6600-42
APC	8817-6600-42
BrdU Staining Buffer Set	**Cat. No.**
BrdU optimized buffers for use with proliferation assays	00-5525-00

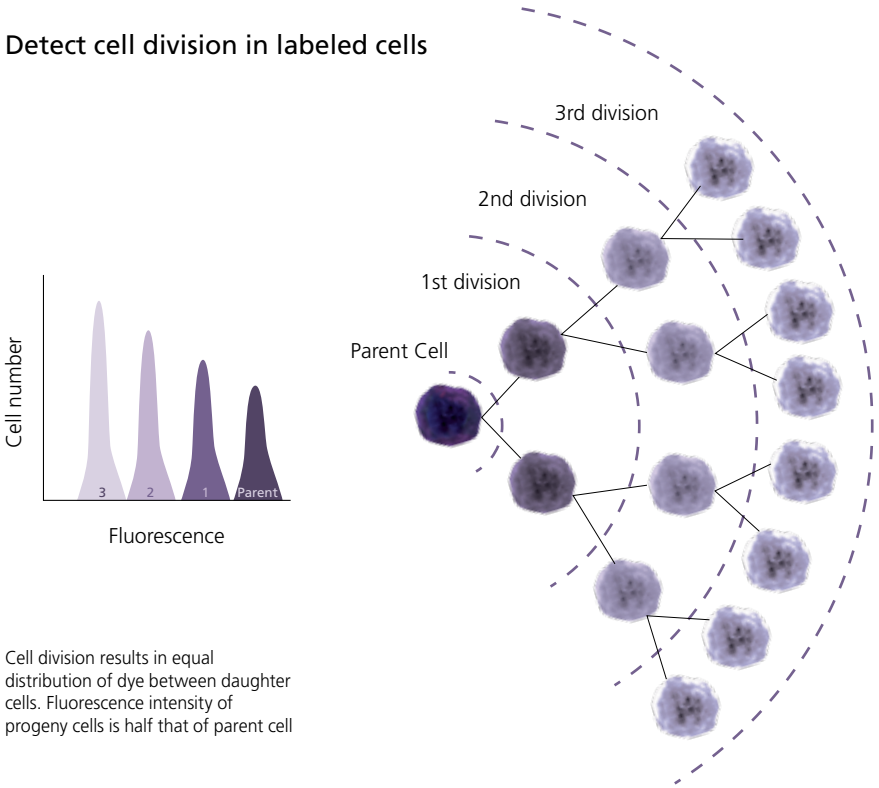
Cell proliferation dyes

The Cell Proliferation Dyes (CPD) eFluor® 670, CPD eFluor® 450 and 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester (CFSE) are fluorescent dyes that can be used to monitor individual cell divisions. Cells labeled with CPD or CFSE may be fixed and permeabilized for analysis of intracellular targets using standard formaldehyde-containing fixatives and saponin-based permeabilization buffers, such as the Foxp3/Transcription Factor Staining Buffer Set (cat. no. 00-5523) or the Intracellular Fixation Buffer (cat. no. 00-8222) and Permeabilization Buffer (cat. no. 00-8222).

CPD bind to cellular proteins containing primary amines. While cells divide, the dye is equally distributed between daughter cells, enabling measurement of successive halving of fluorescence intensity. Between six and eight generations may be visualized, depending on which dye is used. CPD may also be used for long-term tracking of non-dividing, labeled cells.

CFSE readily crosses intact cell membranes reacting with primary amines, crosslinking the dye to intracellular proteins. Cell division is measured as successive halving of CFSE fluorescence intensity, for up to seven generations. CFSE may also be used for long-term tracking of non-dividing, labeled cells.

Detect cell division in labeled cells



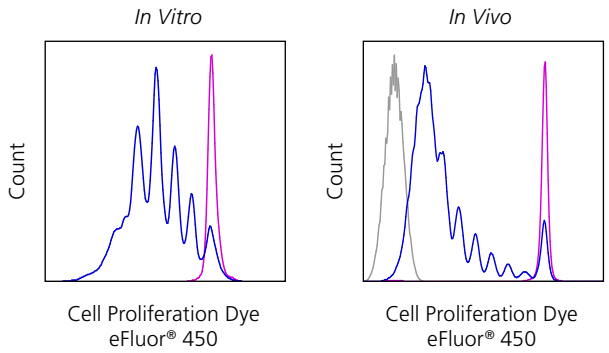
Cell division results in equal distribution of dye between daughter cells. Fluorescence intensity of progeny cells is half that of parent cell

eFluor® Cell Proliferation Dyes

- Excellent dye distibution
- Compatible with GFP
- Lyophilized

eFluor® Cell Proliferation Dyes are membrane permeable fluorescent dyes bound to cellular proteins containing primary amines, which can be used *in vitro* or *in vivo* and visualized for up to 7 generations. Fluorescent dye binds to cellular proteins and is evenly distributed between the daughter cells as they divide. eFluor Cell Proliferation Dyes are supplied in a lyophilized format, that once reconstituted are stable for 6 months when protected from light and stored at -20°C.

Tracking daughter cells



Left: Mouse splenocytes were labeled with 10 uM Cell Proliferation Dye eFluor® 450 and cultured for 3 days with (blue histogram) or without (purple histogram) ConA. Cells were stained with Anti-Mouse CD4 PE (cat. no. 12-0042) and 7-AAD (cat. no. 00-6993). Viable CD4+ cells were used for analysis.

Right: Splenocytes from Thy1.1 mice were labeled with 10 uM Cell Proliferation Dye eFluor® 450 and then injected into C57Bl/6 mice (purple histogram) or B6D2F1 mice (blue histogram). Splenocytes from the C57Bl/6 and B6D2F1 mice were collected 72 hours after injection of the labeled cells. Cells were stained with Anti-Mouse CD4 APC (cat. no. 17-0042), Anti-Mouse/Rat CD90.1 (Thy1.1) PE (cat. no. 12-0900) and Fixable Viability Dye eFluor® 780 (cat. no. 65-0865). Viable Thy1.1+CD4+ cells were used for analysis. Thy1.1-CD4+ host cells from the B6D2F1 mice, that are unlabeled with the Cell Proliferation Dye, are shown in gray.

Cell Proliferation Dyes				
Description	Excitation	Emission	Size	Cat. No.
Violet Laser				
Cell Proliferation Dye eFluor® 450	409 nm	450 nm	500 ug	65-0842-85
			4 x 500 ug	65-0842-90
Blue Laser				
CFSE	488 nm	521 nm	5 x 500 ug	65-0850-84
Red Laser				
Cell Proliferation Dye eFluor® 670	633 nm	670 nm	500 ug	65-0840-85
			4 x 500 ug	65-0840-90

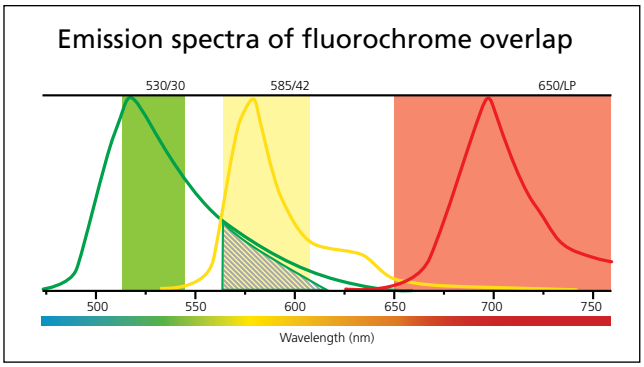
8

Performance Controls

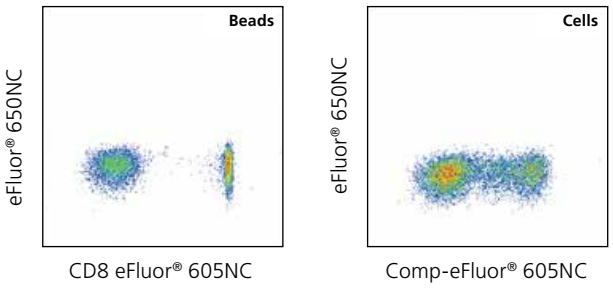
Using controls in your research ensures data accuracy and provides reassurance to review committees, resulting in less chance of paper rejection, faster approval times and greater opportunities to publish. Every laboratory should perform compensation with beads or cells to ensure accurate mean fluorescent intensity is attained, while isotype controls help confirm the specificity of antibody binding.

Compensation overview

Compensation is the process of subtracting spectral spillover from one fluorochrome out of the detector of another, and is generally conducted with cells or beads.

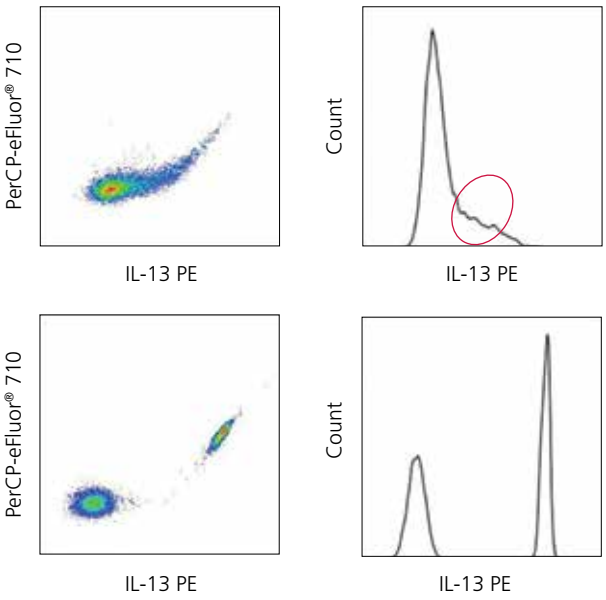


While using cells for compensation can be a good choice, beads are a great alternative when the cell source is limited or the antigen of interest is expressed at low levels or on a rare subpopulation of cells. UltraComp and OneComp eBeads® ensure accurate compensation with a guaranteed negative and positive bead population.



Using cells for compensation can result in difficulty identifying positive and negative cells.

Take the guesswork out of compensation



IL-13 staining on cells

- Broad distribution
- Few positive events
- Difficult to identify positive population

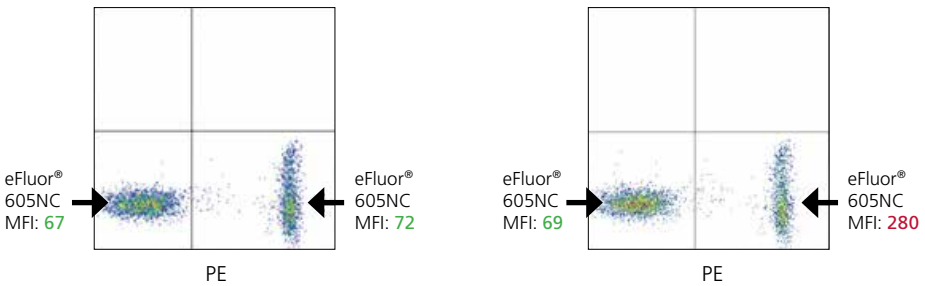
IL-13 staining on OneComp eBeads

- Discrete distribution
- 50% positive
- Easy to identify positive population

Mean Fluorescent Intensity (MFI)

Mean Fluorescent Intensity (MFI) is used in compensation to measure the shift in fluorescent intensity of a population of cells. Fluorescent intensity is determined by comparing negatively stained controls in addition to an antibody. A dim positive stain is slightly brighter than the negative control, whereas the bright stain is generally two logs brighter.

Good compensation requires similar MFI



UltraComp eBeads optimized for the violet laser

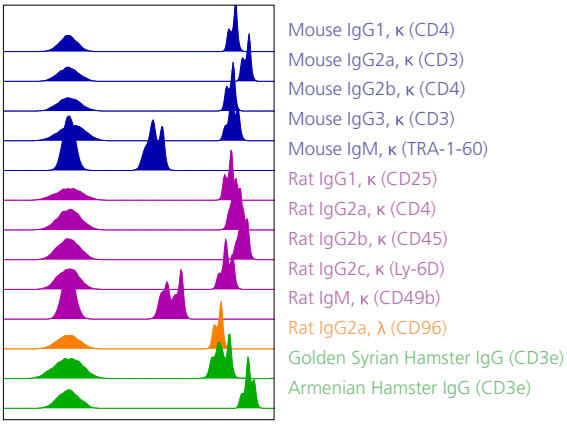
Competitor's compensation beads on the violet laser

Lysed whole blood was stained with CD4 (clone SK3) PE and compensated for spectral spillover of eFluor® 605NC. Performance was compared with a competitor's compensation bead in addition to UltraComp eBeads® which have been optimized for the violet laser.

One vial, one drop approach to compensation

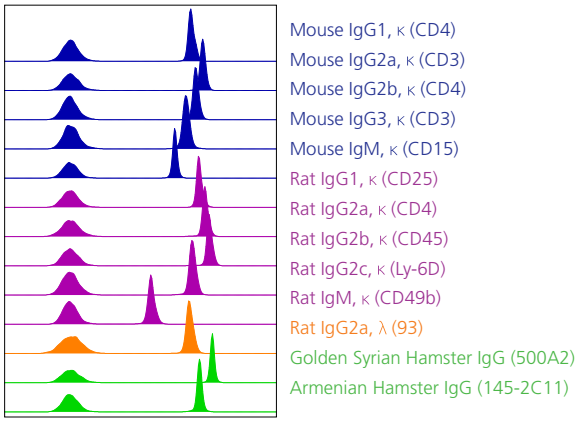
OneComp eBeads® and UltraComp eBeads® are the perfect solution to compensation. They capture mouse, rat and hamster antibodies of IgG and IgM isotypes independent of the light chain, which means they are compatible with almost all antibodies used in flow cytometry. Each vial of compensation eBeads contains a mixture of microspheres that are either coated with capture antibody, resulting in a positive bead, or uncoated, serving as a negative bead. Both beads are combined into one vial and are dispensed as a single drop. Additionally, UltraComp eBeads have been optimized for the violet laser.

OneComp eBeads®



Immunoglobulins PE: Staining of OneComp eBeads with a variety of PE-conjugated monoclonal antibodies.

UltraComp eBeads®



Immunoglobulins eFluor® 450: Staining of UltraComp eBeads with a variety of eFluor® 450 conjugated monoclonal antibodies.

Comparing Compensation Beads

Description	Species Compatibility				Chain Recognition		Features			
	Mouse Ig	Rat Ig	Hamster Ig	Rabbit Ig*	Kappa Light Chain	Lambda Light Chain	One Drop	One Vial	All cell sizes	Violet Laser
UltraComp eBeads® 01-2222	■	■	■	■	■	■	■	■	■	■
OneComp eBeads® 01-1111	■	■	■	■	■	■	■	■	■	
Competitor X Anti-Mouse Ig, k	■				■					
Competitor X Anti-Rat Ig, k		■			■					
Competitor X Anti-Rat/Hamster Ig, k		■	■		■					
Competitor X Plus Anti-Mouse Ig, k	■				■					
Competitor X Plus Anti-Rat Ig, k		■			■					
Competitor Z Anti-Mouse Bead Kit	■				■	■				
Competitor Z Anti-Rat/Hamster Bead Kit		■	■		■	■				

*Rabbit antibodies stain the beads very dimly, but may be useful for some experiments.

UltraComp eBeads® suitable for all lasers

Violet Laser

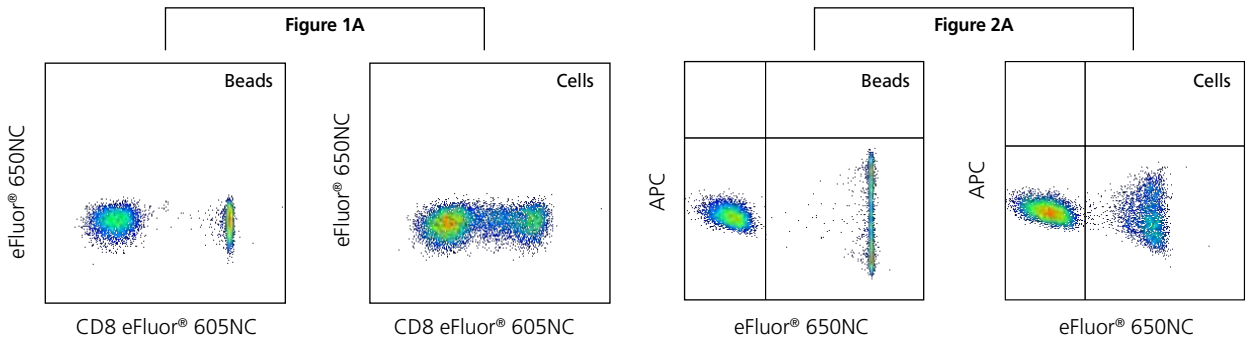


Figure 1A: CD8 eFluor® 605NC is compensated for spectral overlap into the eFluor® 650NC detector using UltraComp eBeads® (left) or cells (right). **Figure 2A:** CD4 eFluor® 650NC is compensated for spectral overlap into the APC detector using UltraComp eBeads® (left) or cells (right).

Blue Laser

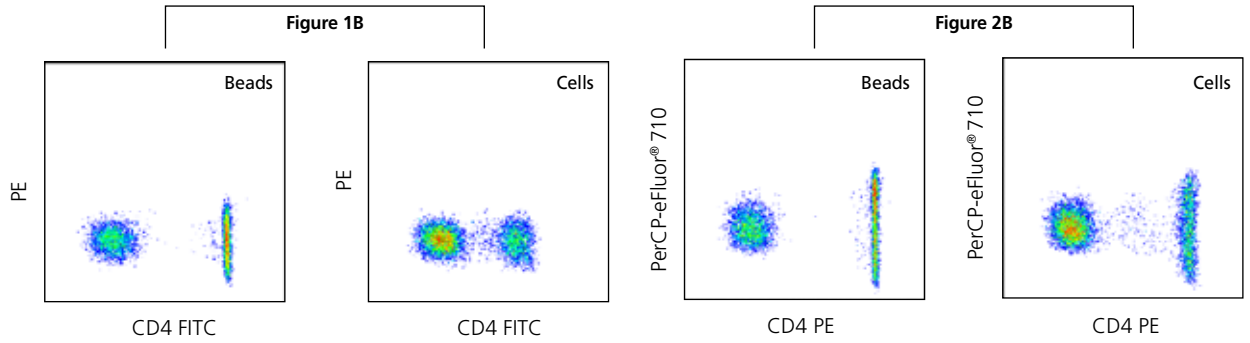


Figure 1B: CD4 FITC is compensated for spectral overlap into the PE detector using UltraComp eBeads® (left) or cells (right). **Figure 2B:** CD4 PE is compensated for spectral overlap into the PerCP-eFluor® 710 detector using UltraComp eBeads® (left) or cells (right).

Red Laser

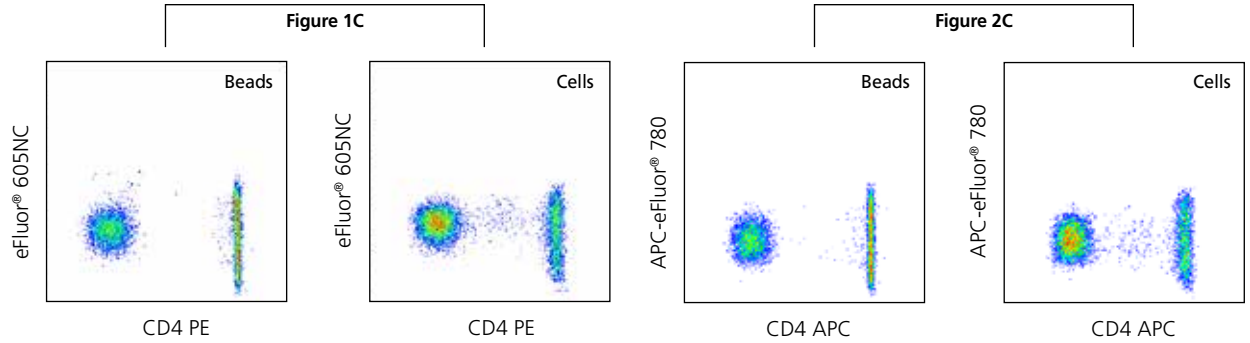


Figure 1C: CD4 PE is compensated for spectral overlap into the eFluor® 605NC detector using UltraComp eBeads® (left) or cells (right). **Figure 2C:** CD4 APC is compensated for spectral overlap into the APC- eFluor® 780 detector using UltraComp eBeads® (left) or cells (right).

Isotype controls

- Confirm specificity of primary antibody binding
- Rule out Fc receptor mediated binding and other non-specific interactions

Selecting the appropriate isotype control is an important element in flow cytometry experiments. Their purpose is to determine background staining and confirm specificity of the experimental antibody. It ideally matches the host species, isotype and conjugation format, in an effort to mimic the non-specific characteristics of the experimental antibodies used.

Isotype controls are developed to assess levels of background staining inherent in cell binding assays. They are a good place to start when optimizing flow cytometer settings and establishing a data range for general autofluorescence from a cell labeled with a conjugated antibody. An isotype control antibody is expected to show low levels of staining on a particular cell population, however sometimes during intracellular staining experiments there may be higher levels of non-specific fluorescence.

There are several potential reasons for inconsistent staining, from inherent differences in the amino acid composition of the two antibodies, to the different amount of fluorophore conjugated to the isotype control versus the experimental antibody, often referred to as fluoroscence-to-protein (F/P) ratio. Activation of cells may also alter the staining patterns of isotype control antibodies. Therefore, using unstimulated cells is recommended, or an inherently negative population in a heterogenous cell preparation as a more relevant negative control, when staining intracellular targets.

Isotype Controls At-a-Glance

Description	Clone	Purified	Functional Grade	Biotin	eFluor® 450	FITC	Alexa Fluor® 488	Cyanine5	PerCP-Cyanine5.5	PE-eFluor® 610	PerCP-eFluor® 710	PE	PE-Cyanine5	PE-Cyanine5.5	PE-Cyanine7	Alexa Fluor® 532	APC	eFluor® 660	Alexa Fluor® 700	APC-eFluor® 780
Mouse IgA		■																		
Mouse IgM	11E10	■	■		■							■					■	■		
Mouse IgM	eMM15	■				■														
Mouse IgG1 κ	P3.6.2.8.1	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■
Mouse IgG2a κ	eBM2a	■	■	■	■	■	■		■		■	■	■		■		■	■	■	■
Mouse IgG2b κ	eBMG2b	■	■	■	■	■	■		■	■	■	■	■	■	■		■	■	■	■
Mouse IgG3		■				■						■								
Rat IgG1 κ	eBRG1	■	■	■	■	■	■	■	■		■	■	■	■	■		■	■	■	■
Rat IgG2a κ	eBR2a	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■
Rat IgG2b κ	eB149/10H5	■	■	■	■	■	■		■		■	■	■	■	■	■	■	■	■	■
Rat IgM		■	■	■	■	■	■			■	■	■					■	■		
Rabbit IgG						■														
Armenian Hamster IgG	eBio299Arm	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■
Golden Syrian Hamster IgG		■	■	■	■	■	■			■	■	■	■		■		■			

Secondary Reagents

Selecting the correct secondary antibody

Although directly conjugated antibodies provide a simple and robust assay detection system, there are situations where a primary antibody followed by a secondary antibody is warranted:

- Primary antibody is not available in a conjugated format
- Primary antibody is not available in the desired format
- Amplification of primary antibody signal is needed

Understanding how to choose the appropriate secondary antibody and format is essential for obtaining the best possible staining results.

Application determines the format

Fluorimetric (fluorochrome-conjugated) secondary antibodies are available in numerous formats, with each specific conjugate determining the application in which the secondary antibody can be used. The variety of available formats provides flexibility, making secondary antibodies versatile across various assay platforms including flow cytometry, immunoassays, immunoblotting and immunohistology.

Host species and isotype of primary determines the secondary antibody

When selecting a secondary antibody it is important to choose one that reacts with the host species of the primary antibody. For example a Goat Anti-Mouse IgG can be used with a mouse primary antibody. Secondary antibodies can be either polyclonal, in which case the host species is typically goat or donkey, or monoclonal where the host species is typically mouse or rat. Additionally, secondary antibodies are available in several classes, either IgA, IgM or IgG, in addition to more specific subclasses such as IgG2a.

Polyclonal antibodies can provide amplification of the signal but must be highly cross-absorbed to provide specificity to the species being stained, and must not react with the other species or other subclasses. Most polyclonal antibodies are F(ab')2 fragmented to minimize Fc binding.

Monoclonal secondaries are typically used when looking at reactivity to a specific IgG subclass. Monoclonal antibodies are consistently reactive to IgG subclasses (IgG1, IgG2a, IgG2b, IgG2c, IgG3), in addition to providing amplification, but to a lesser extent than polyclonal secondaries. Every eBioscience monoclonal antibody has been validated against the specific subclass, as well as lack of reactivity to other subclasses and species. These are useful in multiplexing when using primary antibodies from the same species but different subclasses.

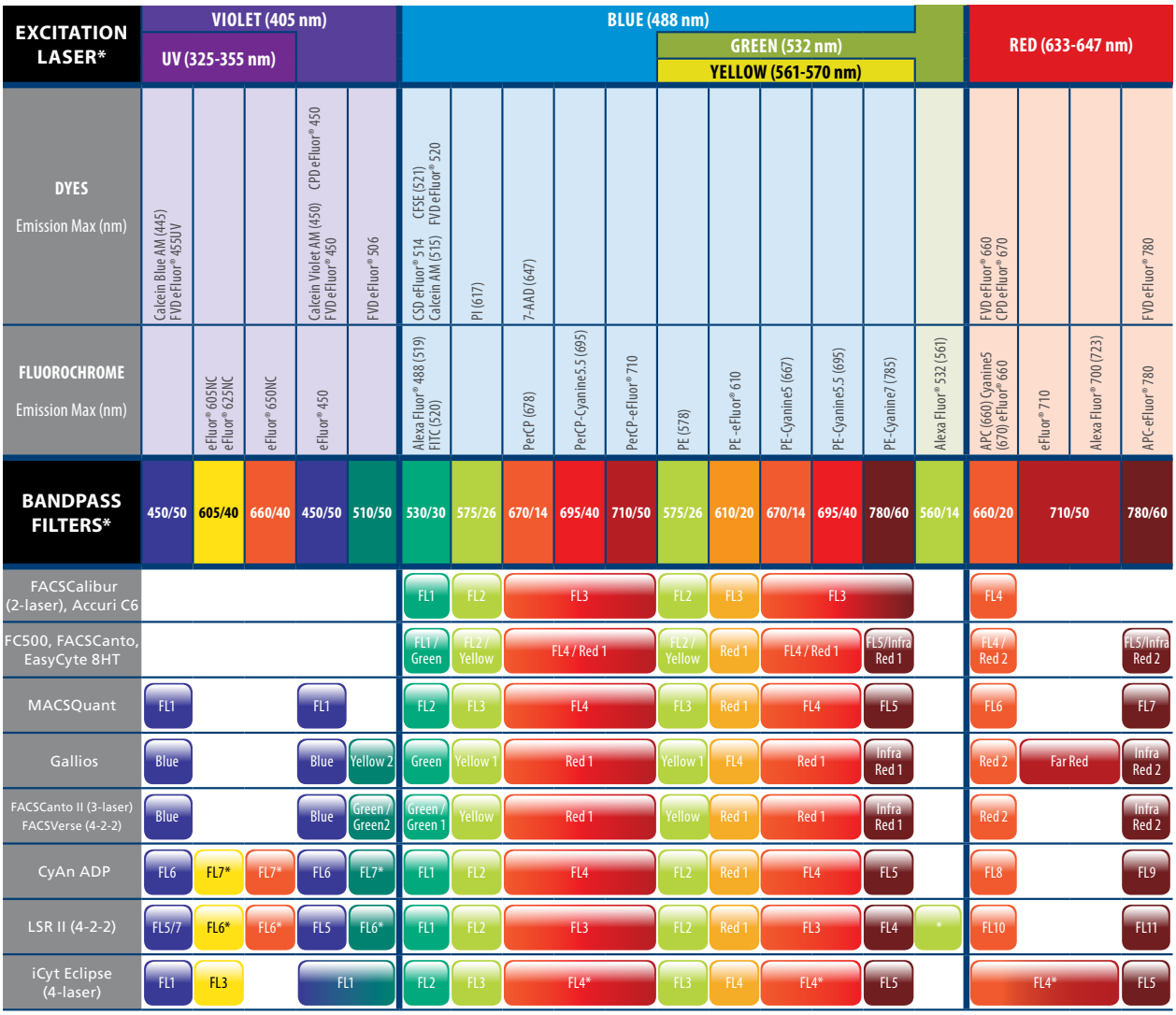
Secondary Antibodies At-a-Glance													
Description	Clone	Cat. Root	Purified	Biotin	eFluor® 450	FITC	PerCP-eFluor® 710	PE	PE-Cyanine5	PE-Cyanine7	eFluor® 660	APC	APC-eFluor® 780
Rat Anti-Mouse IgG	M1-14D12	4015		■	■	■	■	■		■	■	■	
Rat Anti-Mouse IgA	11-44-2	5994		■				■					
Rat Anti-Mouse IgA	mA-6E1	4204				■		■					
Rat Anti-Mouse IgG2a	m2a-15F8	4210		■		■		■				■	
Rat Anti-Mouse IgM	II/41	5790	■	■	■	■	■	■	■	■		■	■
Rat Anti-Mouse IgM	eB121-15F9	5890	■	■	■	■		■					
Mouse Anti-Rat IgM	RM-7B4	4342						■					
Mouse Anti-Rat IgM	HIS40	0990	■			■		■					
Mouse Anti-Rat IgG1	R1-12D10	4812				■						■	
Mouse Anti-Rat IgG2b	R2B-7C3	4815		■				■			■		
Mouse Anti-Rat IgG2c	R2C-23A3	4816						■					
Mouse Anti-Rat IgG2a	r2a-21B2	4817				■	■	■					
Donkey F(ab')2 Anti-Rat IgG	Polyclonal	4822						■				■	
Donkey F(ab')2 Anti-Rabbit IgG	Polyclonal	4739						■					
Goat F(ab')2 Anti-Mouse IgG	Polyclonal	4010				■	■	■			■	■	
Goat Anti-Armenian Hamster IgG	Polyclonal	4111				■							
Goat Anti-Armenian Hamster IgG	Polyclonal	4112						■					
Goat Anti-Golden Syrian Hamster IgG	Polyclonal	4211				■							
Mouse Anti-Biotin	BK-1/39	9895						■					
Mouse Anti-Fluorescein isothiocyanate (FITC)	FITC-9	3300		■							■	■	
Rat Anti-Mouse IgG	Polyclonal	4013		■									
Rat Anti-Mouse IgG2b	m2b-25G4	4220				■							
Rat Anti-Mouse IgE	23G3	5992		■		■	■						
Rat Anti-Mouse IgD	11-26c (11-26)	5993	■	■	■	■	■	■		■		■	
Mouse Anti-Rat IgG		4811				■							
Mouse Anti-Rat IgG	Polyclonal	4813		■									
Goat Anti-Rat IgG	Polyclonal	4818											
Goat Anti-Rat IgG	Polyclonal	4826											
Rat Anti-GFP	5F12.4	6498		■			■				■		
Mouse Anti-GFP	GF28R	6674	■										
Goat F(ab')2 Anti-Rabbit IgG	Polyclonal	4839				■							

Biotin and Streptavidin Conjugates

Biotin is involved in the metabolism of fatty acids, amino acids and gluconeogenesis, however in the laboratory, it can be used to tag molecules of interest for biochemical or cellular studies. While Streptavidin binds to Biotin with high affinity, the fluorochrome conjugates are commonly used with indirect staining protocols to detect biotinylated primary antibodies in flow cytometry. The monoclonal antibody (BK-1/39) specifically recognizes biotin and can be used as an alternative to Streptavidin.

Biotin and Streptavidin			
Description	Clone	Application	Cat. No.
Anti-Biotin Alexa-Fluor® 488	BK-1/39	FC	53-9895
Anti-Biotin PE	BK-1/39	FC	12-9895
Streptavidin eFluor® 450		FC	48-4317
Streptavidin FITC		FC, ICC, IHC	11-4317
Streptavidin PerCP-Cyanine5.5		FC	45-4317
Streptavidin PerCP-eFluor® 710		FC	46-4317
Streptavidin PE		FC	12-4317
Streptavidin PE-Cyanine5		FC	15-4317
Streptavidin PE-Cyanine7		FC	25-4317
Streptavidin APC		FC	17-4317
Streptavidin eFluor® 660		FC, ICC, IHC	50-4317
Streptavidin APC-eFluor® 780		FC	47-4317
Streptavidin Cyanine5		FC	19-4317
Streptavidin eFluor® 710		FC	49-4317

Instrument and fluorochrome/dye chart



Note: Peak emission for eFluor® dyes is noted in the name. Peak emission for all other dyes is shown in parentheses. Before combining reagents in multicolor experiments, always refer to your specific system configuration. *Available lasers, bandpass and longpass filters will vary depending on the configuration.

Key: CSD = Calcium Sensor Dye; CPD = Cell Proliferation Dye; FVD = Fixable Viability Dye; PI = Propidium Iodide

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