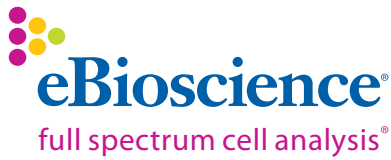


Multicolor Flow Cytometry

Support Products





eBioscience is committed to developing and manufacturing high-quality, innovative reagents in an ISO certified facility. As a provider of more than 10,000 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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Multicolor Flow Cytometry

Support Products

eBioscience is an industry-leading provider of Flow Cytometry antibodies against both novel and well-established targets. With the eFluor® brand of fluorochromes, including eFluor® Nanocrystals, eFluor® Organic Dyes and eFluor® Functional Dyes, eBioscience offers the broadest and most robust selection of fluorochromes available for multicolor flow cytometry. In addition to these innovative fluorochromes, eBioscience also offers a complete set of flow cytometry support products – everything from viability dyes, which help you produce the most accurate data, to staining buffers and secondary antibodies. eBioscience provides a complete solution for your entire flow cytometry workflow.

The eFluor® brand consists of the following product lines:

eFluor® NANOCRYSTALS (NC)

- Direct conjugates for flow cytometry
- Narrow emission spectra and excellent photostability
- Expands the utility of violet laser flow cytometry

eFluor® ORGANIC DYES

- Direct conjugates for flow cytometry
- Robust performance for all work flow scenarios

eFluor® FUNCTIONAL DYES

- Small molecule organic dyes
- Viability dyes for intracellular staining protocols
- Proliferation dyes for tracking cell division
- Fluorescent dyes to monitor intracellular free calcium

Proliferation Tracking Dyes

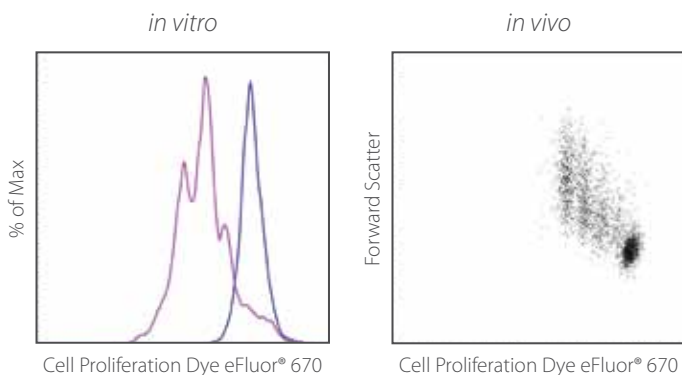
eBioscience provides dyes for analyzing cell proliferation by flow cytometry. With the introduction of Cell Proliferation Dye eFluor® 670, detected in the APC channel, you can now monitor cell division in GFP-expressing cells.

- Cell Proliferation Dye eFluor® 670
- CFSE

Cell Proliferation Dye eFluor® 670

- Can be used in the presence of FITC or GFP
- Can be used both *in vivo* and *in vitro*
- Detects up to six rounds of cell division
- Detected in the APC channel

Cell Proliferation Dye eFluor® 670 is an alternative to CFSE for tracking cell proliferation in situations where CFSE cannot be used due to the presence of FITC or GFP. Cell Proliferation Dye eFluor® 670 emits at 670 nm and is excited with the red laser. As with CFSE, Cell Proliferation Dye eFluor® 670 accurately indicates as many as six rounds of division by partitioning equally into daughter cells during successive cell division and is useful for both *in vitro* and *in vivo* tracking of cell proliferation.



Tracking Cell Proliferation

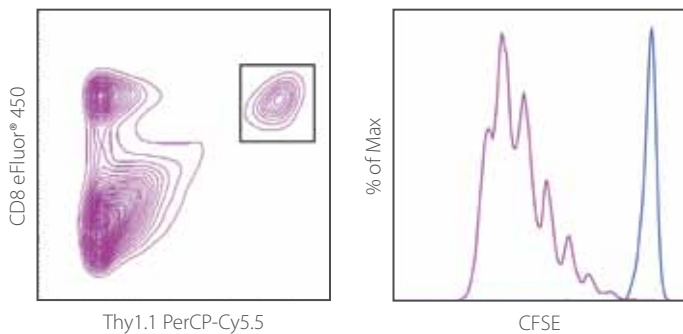
Left: Mouse spleen cells were labeled with 5 μ M Cell Proliferation Dye eFluor® 670, then cultured for three days with (purple histogram) or without (blue histogram) plate-bound anti-mouse CD3 and soluble anti-mouse CD28. Cells were then stained with FITC anti-mouse CD4 (cat. no. 11-0042) and 7-AAD (cat. no. 00-6993). Viable CD4⁺ cells were used for analysis.

Right: C57Bl/6 spleen cells were labeled with 5 μ M Cell Proliferation Dye eFluor® 670, then injected into C57Bl/6 x DBA/2 F1 mice. Two days later, spleen cells from the F1 mice were stained with PE-Cy7 anti-mouse CD4 (cat. no. 25-0042) and Fixable Viability Dye eFluor® 450 (cat. no. 65-0863). Viable CD4⁺ cells were used for analysis.

CFSE

- Gold standard in cell proliferation analysis
- Use for tracking cell division both *in vitro* & *in vivo*
- Detected in the FITC channel

CFSE is the gold standard for analyzing cell proliferation and has also been used in CTL assays and cell motility studies. CFSE readily crosses intact cell membranes. Once inside the cells, intracellular esterases cleave the acetate groups to yield the fluorescent carboxyfluorescein molecule. The succinimidyl ester group reacts with primary amines, crosslinking the dye to intracellular proteins. Cell division can be measured as successive halving of the fluorescence intensity of CFSE.



Tracking Cell Proliferation *in vitro*

T-cell receptor transgenic, OVA-reactive OT-I cells undergo several rounds of cell division in response to OVA, but not PBS, *in vivo*. Lymph node cells from OT-I mice were labeled with 1 μ M CFSE (cat. no. 65-0850) and adoptively transferred into C57Bl/6 mice. Mice were then immunized with OVA or PBS. Three days later, splenocytes were stained with PerCP-Cy5.5 anti-mouse/rat Thy1.1 and eFluor® 450 anti-mouse CD8 to gate on the OT-I cells (left). Cells in the OT-I gate (CD8+Thy1.1+) were analyzed for cell division (right), measured as discrete peaks of decreasing fluorescence of CFSE in OVA-immunized mice (purple histogram), or undivided cells with a single, bright CFSE peak in PBS-immunized mice (blue histogram).

Proliferation Tracking Dyes Ordering Information

Description	Catalog Number	Sizes	Excitation Laser	Detector	Peak Emission (nm)
CFSE	65-0850-84	1 Pack (5 X 500 μ g)	Blue (488 nm)	FITC	521
Cell Proliferation Dye eFluor® 670	65-0840-90	1 Pack (5 X 400 μ g)	Red (633 nm)	APC	670

Cell Viability Dyes

eBioscience offers a variety of solutions for assessing cell viability. Our multiple viability dyes provide flexibility when designing your multicolor staining panel. Fixable Viability eFluor® Dyes can be used to determine viability in both surface and intracellular flow cytometry staining protocols.

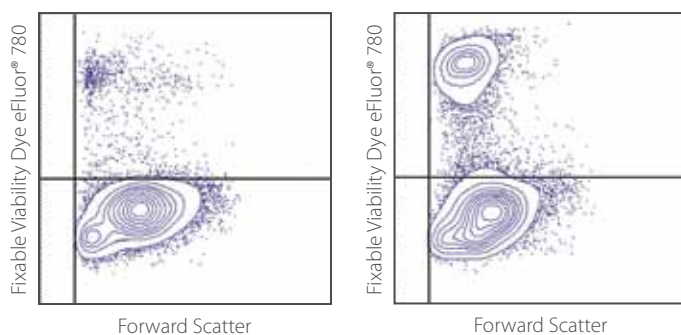
- **Fixable Viability Dye eFluor® 450, 660 and 780**
- **Calcein AM, Calcein Blue AM, Calcein Violet 450 AM**
- **Propidium Iodide & 7-AAD**

Fixable Viability eFluor® Dyes

- Assess viability of cells when staining both surface and intracellular targets
- Assess viability without intercalating DNA
- Signal maintained in cryopreserved samples
- Available for the violet and red lasers
- Provided in convenient 1 µl test size

eBioscience Fixable Viability eFluor® Dyes discriminate live cells from dead cells, yet are fully compatible with intracellular staining protocols. Unlike 7-AAD and Propidium Iodide, Fixable Viability eFluor® Dyes are retained by dead cells throughout fixation, permeabilization and even cryopreservation protocols. Using Fixable Viability eFluor® Dyes with violet and red lasers is easy; simply label cells prior to antibody staining, then follow normal procedures. The result is a brightly fluorescent dead cell population which can be excluded during data analysis, thereby improving data quality.

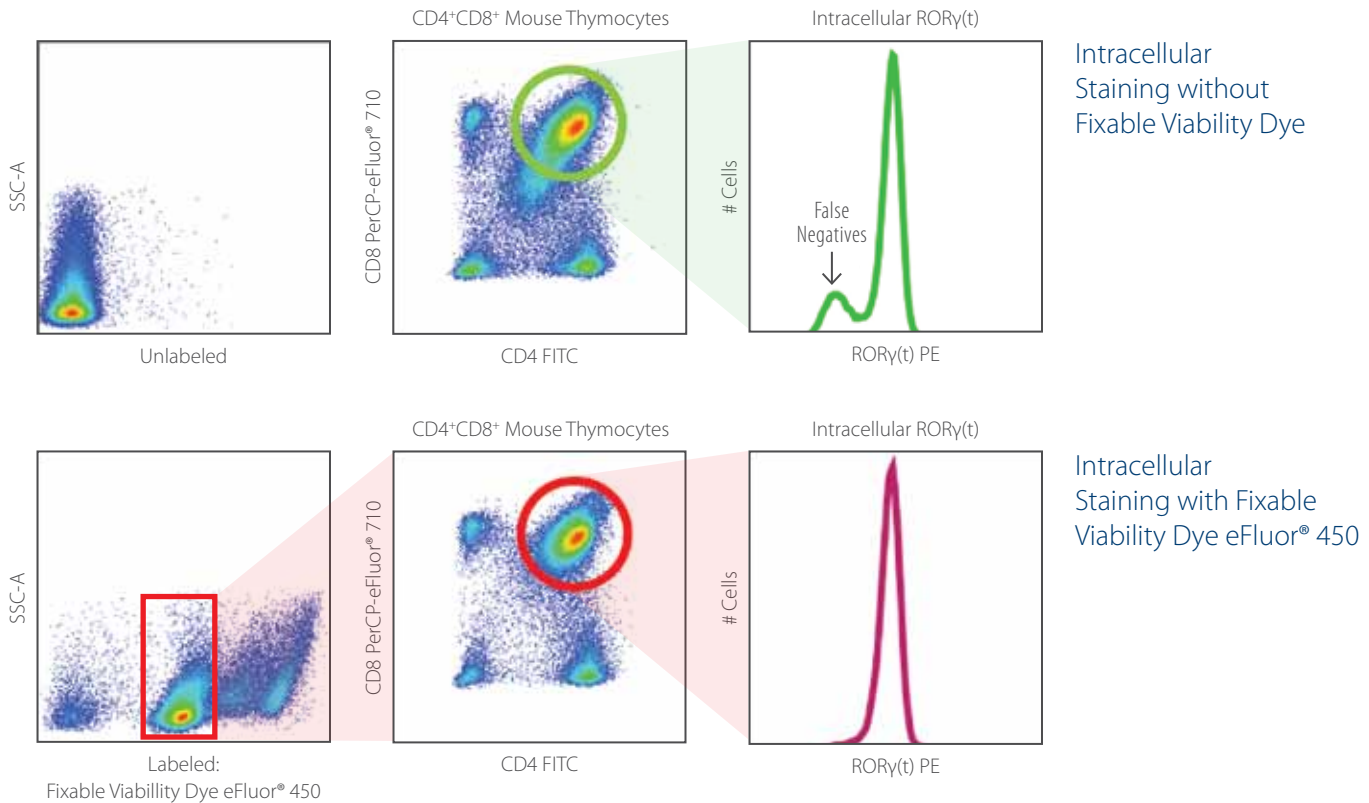
Note: Excluding dead cells from analysis is a recommended procedure for all intracellular staining protocols including detection of nuclear factors, transcription factors, cytosolic signaling molecules as well as secreted growth factors and cytokines.



Detection of Viable Cells in Surface Staining

BALB/c thymocytes were uncultured (left) or cultured overnight at 37°C (right) and then stained with 1 µl of Fixable Viability Dye eFluor® 780 (cat. no. 65-0865) per ml of thymocytes that were resuspended at 5x10⁶/ml in PBS. Total cells were used for analysis.

Detection of Viable Cells in Intracellular Staining



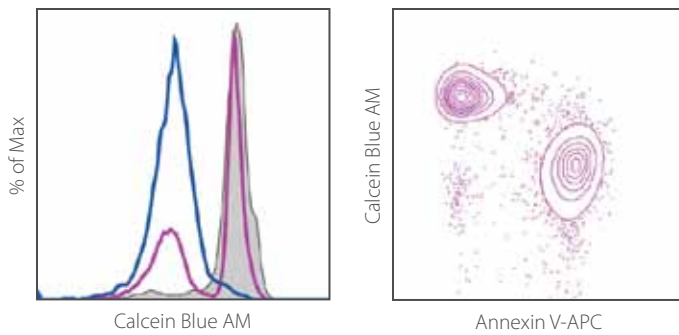
eFluor[®] Fixable Viability Dye Ordering Information

Description	Catalog Number	Sizes	Excitation Laser	Peak Emission (nm)
Fixable Viability Dye eFluor [®] 450	65-0863-14	100 tests	Violet (405 nm)	450
	65-0863-18	500 tests (5 X 100 tests)		
Fixable Viability Dye eFluor [®] 660	65-0864-14	100 tests	Red (633 nm)	660
	65-0864-18	500 tests (5 X 100 tests)		
Fixable Viability Dye eFluor [®] 780	65-0865-14	100 tests	Red (633 nm)	780
	65-0865-18	500 tests (5 X 100 tests)		

Calcein AM, Calcein Blue AM, Calcein Violet 450 AM

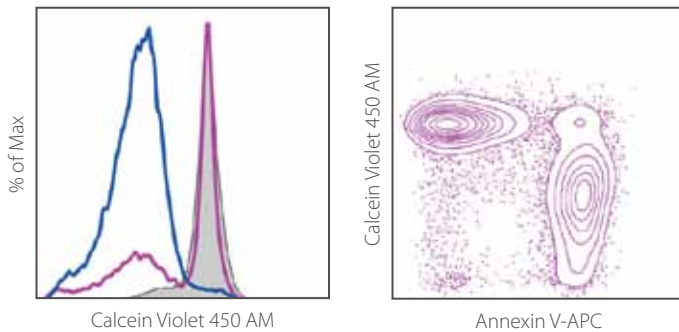
Calcein labeling dyes easily cross the cell membrane and selectively label live cells for analysis by flow cytometry or fluorescence microscopy. Calcein AM is non-toxic and may be used for short-term cell tracing. Calcein Blue AM, a UV excited alternative to Calcein AM, has excitation characteristics similar to DAPI, Hoechst, and AMCA dyes. Calcein Violet 450 AM is a violet laser (405 nm) equivalent to Calcein AM.

Co-staining with Annexin V or 7-AAD is recommended to allow the greatest resolution between live and dead/apoptotic cells.



Calcein Blue AM

Balb/c thymocytes were stained with 1 μ M Calcein Blue AM (cat. no. 65-0855) 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 μ M 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.



Calcein Violet 450 AM

Balb/c thymocytes were stained with 1 μ M 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 μ M 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.

Calcein Labeling Dyes Ordering Information

Description	Catalog Number	Sizes	Excitation Laser	Peak Emission (nm)
Calcein, AM	65-0853-39	1 mg	Blue (488 nm)	515
	65-0853-78	1 Pack (20 X 50 μ g)		
Calcein Blue, AM	65-0855-39	1 mg	UV (355 nm)	445
Calcein Violet 450, AM	65-0854-39	1 mg	Violet (405 nm)	450

Propidium Iodide & 7-AAD

eBioscience offers Propidium Iodide (PI) and 7-amino-actinomycin D (7-AAD) for assessing cell viability by flow cytometry. Both dyes are available as ready-to-use solutions for the exclusion of nonviable cells in flow cytometric analysis. PI binds to double stranded DNA of dead cells and is excluded from cells with intact plasma membranes. PI can be detected with the PerCP tandem dye channel for viability exclusion. Analyze PI in the PE channel when used as a counterstain for FITC-Annexin V. 7-AAD can be used in place of PI. Fluorescence is detected in the far red range of the spectrum (650 nm long-pass filter). PI and 7-AAD can also be used for flow cytometric analysis of the cell cycle.

Propidium Iodide & 7-AAD Ordering Information

Description	Catalog Number	Sizes	Excitation Laser	Peak Emission (nm)
Propidium Iodide (PI)	00-6990-50	2 ml	Blue (488 nm)	617
7-AAD	65-6993-50	2 ml	Blue (488 nm)	647

Improve Your Flow Data

- Block non-specific Fc-mediated binding of antibodies
- Reduce background and improve resolution of flow cytometry data

eBioscience Fc-Blocking reagents are used to inhibit the non-specific Fc-gamma receptor (FcγR)-mediated binding of antibodies used for flow cytometric analysis. Blocking non-specific Fc fragment-mediated binding of antibodies to cell surface Fc receptors is necessary during staining for flow cytometry for optimal staining and signal to noise ratio. eBioscience provides reagents for blocking Fc-binding in both mouse and human cells. Four different classes of Fcγ receptors have been identified and are expressed at varying levels in multiple cell lineages with high expression in myeloid, granulocyte and B cells. The extent to which monoclonal antibodies will bind to FcγR varies depending on the isotype of the monoclonal antibody. Furthermore, different monoclonal antibodies of the same isotype will display differential binding to FcγRs.

Fc-Blocking Reagents Ordering Information

Description	Clone	Isotype	Format	Size	Catalog Number
Human FcγR-Binding Inhibitor	--	--	Purified	25 tests	14-9161-71
	--	--	Purified	100 tests	14-9161-73
	--	--	Functional Grade	25 tests	16-9161-71
	--	--	Functional Grade	100 tests	16-9161-73
anti-mouse CD16/32 - Blocks Fc - Binding	93	Rat IgG2a, λ	Purified	50 µg	14-0161-81
	93	Rat IgG2a, λ	Purified	100 µg	14-0161-82
	93	Rat IgG2a, λ	Purified	500 µg	14-0161-85
	93	Rat IgG2a, λ	Purified	1 mg	14-0161-86
	93	Rat IgG2a, λ	Functional Grade	50 µg	16-0161-81
	93	Rat IgG2a, λ	Functional Grade	100 µg	16-0161-82
	93	Rat IgG2a, λ	Functional Grade	500 µg	16-0161-85
	93	Rat IgG2a, λ	Functional Grade	1 mg	16-0161-86

Flow Cytometry Buffers & Solutions

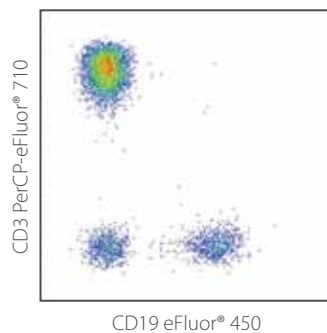
Optimal detection of cellular antigens by flow cytometry requires the use of optimized staining buffers. eBioscience provides appropriate staining and cell preparation buffers regardless of whether your target is secreted, nuclear, or on the cell surface.

1-Step Fix/Lyse Solution (10X)

eBioscience 1-Step Fix/Lyse Solution simplifies your analysis of human blood. This solution lyses red blood cells (RBCs) after staining peripheral blood with fluorochrome conjugated antibodies, and leaves behind stained and fixed leukocytes, all in one step.

1-Step Fix/Lyse Solution Advantages Include:

- RBC lysis and fixation of samples in only 15 minutes
- Allows temporary storage of fixed and stained samples
- Eliminates the need for gradient centrifugation separation



1-Step Fix/Lyse Solution (10x)

Normal human peripheral blood cells were stained with FITC anti-human CD45, PerCP-eFluor® 710 anti-human CD3, and eFluor® 450 anti-human CD19, and then incubated with eBioscience 1-Step Fix/Lyse Solution (cat. no. 00-5333) for 15 minutes at room temperature. Cells were centrifuged, washed once in flow staining buffer, and 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 cells gated on CD45+ events.



New products are launched regularly. [Discover more at www.eBioscience.com.](http://www.eBioscience.com)

eBioscience Intracellular Staining Buffers

When performing intracellular staining for flow cytometry the selection of buffers used for fixation and permeabilization has a significant impact on the quality and accuracy of data. eBioscience provides optimized buffer solutions for nuclear factors, transcription factors, and cytosolic and secreted proteins. The table below summarizes which buffer system is appropriate for target antigens in various cellular locations.

Intracellular Staining Buffer Selection Guide

Target Antigen	eBioscience Foxp3 Staining Buffer Set (cat. no. 00-5523)	eBioscience Fixation & Permeabilization Kit (cat. no. 88-8823)
Transcription Factors	Yes	No
Nuclear Proteins	Yes	Not tested
Cytokines (Secreted Proteins)	Yes†	Yes
Cytoplasmic Proteins	Not tested	Yes

† Validated for most cytokine proteins

Flow Cytometry Buffers & Solutions Ordering Information

Description	Size	Catalog Number
Brefeldin A Solution	1 ml	00-4506-51
Monensin Solution	1 ml	00-4505-51
Flow Cytometry Staining Buffer	600 ml	00-4222-26
	200 ml	00-4222-57
eFluor® NC Flow Cytometry Staining Buffer	200 ml	00-3222-57
IC Fixation Buffer	125 ml	00-8222-49
Permeabilization Buffer	100 ml	00-8333-56
Fixation & Permeabilization Kit	Kit	88-8823-88
Foxp3 Fixation/Permeabilization Concentrate	30 ml	00-5123-43
Foxp3 Fixation/Permeabilization Diluent	100 ml	00-5223-56
Foxp3 Fixation/Permeabilization Concentrate and Diluent	--	00-5521-00
Foxp3 Staining Buffer Set	--	00-5523-00
Red Blood Cell Lysis Buffer (1X)	200 ml	00-4333-57
1-Step Fix/Lyse Solution (10X)	50 ml	00-5333-54
	200 ml	00-5333-57

Second Step Reagents

eBioscience provides a complete set of secondary reagents, reactive with either heavy or light chains of various antibody isotypes, and conjugated to our complete panel of fluorochromes.

Second Step Reagents Ordering Information

Reactivity	Clone	Host	Isotype	Catalog Number	Format	Size
Hamster IgG, Armenian	polyclonal	Goat	--	11-4111-85	FITC	500 µg
				13-4113-85	Biotin	500 µg
				12-4112-83	PE	200 µg
Hamster IgG, Syrian	polyclonal	Goat	--	13-4213-85	Biotin	500 µg
				11-4211-85	FITC	500 µg
Human IgG Fcγ Fragment Specific	polyclonal	Goat	--	12-4998-82	PE	100 µg
				13-4998-83	Biotin	200 µg
Human IgL, κ	HP6062	Mouse	IgG1, k	13-9997-80	Biotin	25 µg
				13-9997-82	Biotin	100 µg
				14-9997-80	Purified	25 µg
				14-9997-82	Purified	100 µg
Human IgL, λ	JDC-12	Mouse	IgG1, k	13-9996-80	Biotin	25 µg
				13-9996-82	Biotin	100 µg
				14-9996-80	Purified	25 µg
				14-9996-82	Purified	100 µg
Human IgM	SA-DA4	Mouse	IgG1, k	13-9998-80	Biotin	25 µg
				13-9998-82	Biotin	100 µg
				14-9998-80	Purified	25 µg
				14-9998-82	Purified	100 µg
Mouse F(ab') ₂ Fragment IgG (Heavy & Light Chain)	polyclonal	Donkey	IgG	12-4012-82	PE	100 µg
				17-4012-82	APC	100 µg
				12-4012-87	PE	250 µg
Mouse IgA	11-44-2	Rat	IgG1, k	12-5994-81	PE	50 µg
				12-5994-82	PE	100 µg
				12-5994-83	PE	200 µg
				13-5994-81	Biotin	50 µg
				13-5994-82	Biotin	100 µg
				13-5994-85	Biotin	500 µg
				14-5994-81	Purified	50 µg
				14-5994-82	Purified	100 µg
14-5994-85	Purified	500 µg				

Second Step Reagents Ordering Information (continued)

Reactivity	Clone	Host	Isotype	Catalog Number	Format	Size
Mouse IgA	mA-6E1	Rat	IgG1, k	11-4204-81	FITC	50 µg
				11-4204-82	FITC	100 µg
				11-4204-83	FITC	200 µg
				12-4204-81	PE	50 µg
				12-4204-82	PE	100 µg
				12-4204-83	PE	200 µg
Mouse IgD	11-26c (11-26)	Rat	IgG2a, k	11-5993-81	FITC	50 µg
				11-5993-82	FITC	100 µg
				11-5993-85	FITC	500 µg
				12-5993-81	PE	50 µg
				12-5993-82	PE	100 µg
				12-5993-83	PE	200 µg
				13-5993-81	Biotin	50 µg
				13-5993-82	Biotin	100 µg
				13-5993-85	Biotin	500 µg
				14-5993-81	Purified	50 µg
				14-5993-82	Purified	100 µg
				14-5993-85	Purified	500 µg
				48-5993-80	eFluor® 450	25 µg
				48-5993-82	eFluor® 450	100 µg
				51-5993-80	Alexa Fluor® 647	25 µg
51-5993-82	Alexa Fluor® 647	100 µg				
Mouse IgE	23G3	Rat	IgG1, k	11-5992-81	FITC	50 µg
				11-5992-82	FITC	100 µg
				11-5992-85	FITC	500 µg
				12-5992-81	PE	50 µg
				12-5992-82	PE	100 µg
				12-5992-83	PE	200 µg
				13-5992-81	Biotin	50 µg
				13-5992-82	Biotin	100 µg
				13-5992-85	Biotin	500 µg
				14-5992-81	Purified	50 µg
				14-5992-82	Purified	100 µg
				14-5992-85	Purified	500 µg
Mouse IgG	polyclonal	Rat	--	11-4011-85	FITC	500 µg
				13-4013-85	Biotin	500 µg

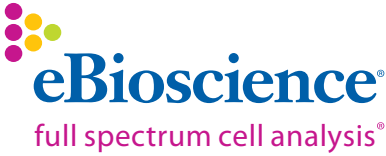
Second Step Reagents Ordering Information (continued)

Reactivity	Clone	Host	Isotype	Catalog Number	Format	Size			
Mouse IgM	II/41	Rat	IgG2a, k	11-5790-81	FITC	50 µg			
				11-5790-82	FITC	100 µg			
				11-5790-85	FITC	500 µg			
				12-5790-81	PE	50 µg			
				12-5790-82	PE	100 µg			
				12-5790-83	PE	200 µg			
				13-5790-81	Biotin	50 µg			
				13-5790-82	Biotin	100 µg			
				13-5790-85	Biotin	500 µg			
				14-5790-81	Purified	50 µg			
				14-5790-82	Purified	100 µg			
				14-5790-85	Purified	500 µg			
				15-5790-81	PE-Cy5	50 µg			
				15-5790-82	PE-Cy5	100 µg			
				17-5790-82	APC	100 µg			
				25-5790-81	PE-Cy7	50 µg			
				25-5790-82	PE-Cy7	100 µg			
				35-5790-81	PE-Cy5.5	50 µg			
				35-5790-82	PE-Cy5.5	100 µg			
				36-5790-85	Functional Grade - Biotin	500 µg			
				eB121-15F9	Rat	IgG2a, k	11-5890-81	FITC	50 µg
							11-5890-82	FITC	100 µg
							11-5890-85	FITC	500 µg
							12-5890-81	PE	50 µg
	12-5890-82	PE	100 µg						
	12-5890-83	PE	200 µg						
	13-5890-81	Biotin	50 µg						
	13-5890-82	Biotin	100 µg						
	13-5890-85	Biotin	500 µg						
	14-5890-81	Purified	50 µg						
	14-5890-82	Purified	100 µg						
	14-5890-85	Purified	500 µg						
	Rabbit F(ab') ₂ Fragment IgG (Heavy & Light Chain)	polyclonal	Donkey	--	12-4739-81	PE	50 µg		
			Goat	--	11-4839-81	FITC	50 µg		

Second Step Reagents Ordering Information (continued)

Reactivity	Clone	Host	Isotype	Catalog Number	Format	Size
Rat F(ab') ₂ Fragment IgG (Heavy & Light Chain)	polyclonal	Donkey	--	12-4822-82	PE	100 µg
				17-4822-82	APC	100 µg
				12-4822-87	PE	250 µg
Rat IgG	polyclonal	Mouse	--	11-4811-85	FITC	500 µg
				13-4813-85	Biotin	500 µg
Rat IgG1	R1-3G1	Mouse	IgG1, k	11-4814-85	FITC	500 µg
Rat IgM	HIS40	Mouse	IgG1, k	11-0990-82	FITC	100 µg
				12-0990-82	PE	100 µg
				14-0990-82	Purified	100 µg
Rat IgG (γ-chain specific)	polyclonal	Goat	--	26-4826-82	TRITC	100 µg
Streptavidin	--	--	--	11-4317-87	FITC	250 µg
				12-4317-87	PE	250 µg
				15-4317-82	PE-Cy5	100 µg
				17-4317-82	APC	100 µg
				19-4317-82	CY5	100 µg
				25-4317-82	PE-Cy7	100 µg
				30-4317-82	APC-Cy5.5	100 µg
				35-4317-82	PE-Cy5.5	100 µg
				45-4317-80	PerCP-Cy5.5	25 µg
				45-4317-82	PerCP-Cy5.5	100 µg
				47-4317-82	APC-eFluor® 780	100 µg
				48-4317-82	eFluor® 450	100 µg
				93-4317-41	eFluor® 605NC	25 tests
				93-4317-42	eFluor® 605NC	100 tests
				94-4317-71	eFluor® 625NC	25 tests
				94-4317-73	eFluor® 625NC	100 tests
				95-4317-41	eFluor® 650NC	25 tests
				95-4317-42	eFluor® 650NC	100 tests

Note: eFluor® 450 is a replacement for Pacific Blue®. APC-eFluor® 780 is a replacement for APC-Alexa Fluor® 750.



Headquarters

eBioscience, Inc.
10255 Science Center Drive
San Diego, CA 92121
USA

SERVICE AND SUPPORT FOR DIRECT SALES

North America

Technical Support:
For Research Products:
888.810.6168
858.642.2058
tech@eBioscience.com
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858.642.2058
tech@eBioscience.com
Customer Service:
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858.642.2058
info@eBioscience.com
Fax:
858.642.2046

Austria

Technical Support:
tech@eBioscience.com
Customer Service:
+43 1 796 40 40-305
Austria@eBioscience.com
Fax:
+43 1 796 40 40-400

Belgium

Technical Support:
tech@eBioscience.com
Customer Service:
+43 1 796 40 40 - 308
Belgium@eBioscience.com
Fax:
+43 1 796 40 40 - 400

France

Technical Support:
tech@eBioscience.com
Customer Service:
0 800 800 417
France@eBioscience.com
Fax:
0 800 800 418

Germany

Technical Support:
tech@eBioscience.com
Customer Service:
069 33 29 64 56
Germany@eBioscience.com
Fax:
069 255 77 335

Ireland

Technical Support:
tech@eBioscience.com
Customer Service:
+44 208 951 4482
Ireland@eBioscience.com
Fax:
+44 208 952 7527

Netherlands

Technical Support:
tech@eBioscience.com
Customer Service:
+43 1 796 40 40 - 308
Netherlands@eBioscience.com
Fax:
+43 1 796 40 40 - 400

Switzerland

Technical Support:
tech@eBioscience.com
Customer Service:
+41 21 510 1214
Switzerland@eBioscience.com
Fax:
+41 21 510 1216

United Kingdom

Technical Support:
tech@eBioscience.com
Customer Service:
+44 208 951 4482
UK@eBioscience.com
Fax:
+44 208 952 7527

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