BD Pharmingen™

BrdU Flow Kits

Instruction Manual

FITC BrdU Flow Kit

Cat. No. 559619 (50 tests)
Cat. No. 557891 (4 × 50 tests)

APC BrdU Flow Kit

Cat. No. 552598 (50 tests)
Cat. No. 557892 (4 × 50 tests)

BD

Kit Contents

For catalog numbers 559619 and 552598:

(Store the following items at 4°C)

- Fluorochrome-conjugated Anti-BrdU Antibody: 1 vial.
- BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution: 1 vial.
- BD Perm/Wash™ Buffer (10×): 2 vials.
- BD Cytoperm™ Plus Permeabilization Buffer: 1 vial.
- 7-AAD: 1 vial.
- Kit Manual

PLEASE NOTE:

(These items are shipped separately and should be stored at –80°C)

- BrdU: 5 vials.
- DNase: 5 vials.

Catalog numbers 557891 and 557892 are made up of four individual kits as described above.

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Introduction

The immunofluorescent staining of incorporated bromodeoxyuridine (BrdU) and flow cytometric analysis provide a high resolution technique to determine the frequency and nature of individual cells that have synthesized DNA. In this method, BrdU (an analog of the DNA precursor thymidine) is incorporated into newly synthesized DNA by cells entering and progressing through the S (DNA synthesis) phase of the cell cycle.[1-4] The incorporated BrdU is stained with specific anti-BrdU fluorescent antibodies. The levels of cell-associated BrdU are then measured by flow cytometry. Often, staining with a dye that binds to total DNA such as 7-amino-actinomycin D (7-AAD) is coupled with immunofluorescent BrdU staining. With this combination, two-color flow cytometric analysis permits the enumeration and characterization of cells that are actively synthesizing DNA (BrdU incorporation) in terms of their cell cycle position (ie, G0/1, S, or G2/M phases defined by 7-AAD staining intensities).[5, 6]

Prolonged exposure of cells to BrdU allows for the identification and analysis of actively cycling, as opposed to non-cycling, cell fractions. Pulse labeling of cells with BrdU at various time points, permits the determination of cell-cycle kinetics. It should be noted that BrdU incorporation studies have been used in a variety of experimental protocols. These include *in vitro* and *in vivo* (eg, intraperitoneal injection or administration via drinking water) labeling systems.

An important feature of the BD Pharmingen™ BrdU Flow Kit is that it provides reagents for immunofluorescent BrdU staining with a protocol that is compatible with the use of additional fluorescent antibodies specific for other cellular molecules. These molecules may include cell surface antigens or intracellular proteins (eg, cytokines, cyclins, and other proteins) whose expression or activity may be related to the cell's activation, entry and progression through cell cycle or cell death. This is possible because the BrdU Flow Kit staining protocol avoids DNA-denaturing agents such as acid, ethanol, and high temperatures that can result in altered cellular light-scattering characteristics and limit the recognition of cellular antigens by fluorescent antibodies.[7-11]

Fluorescent antibodies that are capable of recognizing cell surface antigens or proteins in cells that have been fixed with paraformaldehyde and permeabilized with saponin can be used with the BrdU Flow Kit. With this combination of reagents, the expression levels of various surface or intracellular proteins can be measured by flow cytometry relative to the cell's DNA synthetic activity (BrdU incorporation level). For example, the BrdU Flow Kit can be used with fluorescent anti-cytokine antibodies in time course analyses of cultured cells following *in vitro* mitogenic stimulation of quiescent lymphoid cell populations. In this way, the levels of a particular cytokine (eg, the T cell growth and differentiation factor, IL-2) that are expressed prior to, at, and following the onset of DNA synthesis (during the first major round of cell-cycle activity) can be studied.

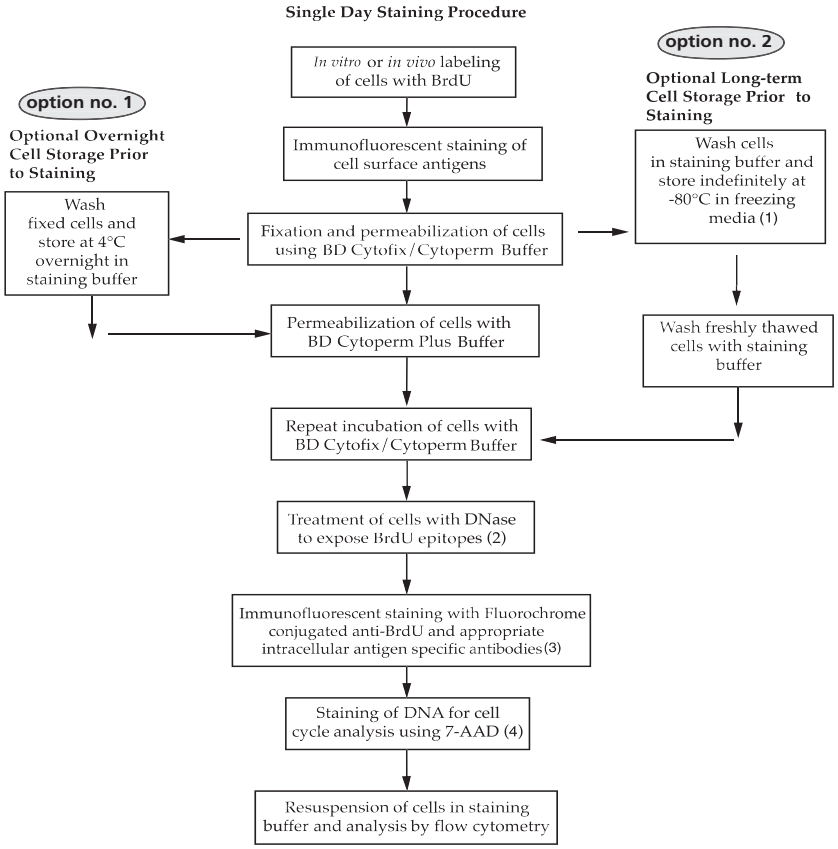
Many high-resolution studies of this type are possible with the use of the BD Pharmingen BrdU Flow Kit and other selected flow cytometry reagents. The kit ensures consistent results by providing detailed instructions and all critical reagents necessary to implement the staining protocol. These individual components have been rigorously tested for their suitability to perform multiparameter analyses

of incorporated BrdU levels, cell surface antigen expression, and expression of intracellular antigens by individual cells. BrdU uptake can also be analyzed in frozen or paraffin embedded tissue sections. The BD Pharmingen™ BrdU *In-Situ* Kits (Cat. Nos. 550803, 551321) provide reagents which allow for two-color staining in tissue sections.

Overview: The BD Pharmingen™ BrdU Flow Kit Staining Protocol

The BD Pharmingen™ BrdU Flow Kit staining procedure offers several options for sample handling. With this staining protocol, it is possible to immunofluorescently stain and analyze samples in a single day. The entire staining procedure requires approximately 3 hours. Alternatively, samples may be fixed and stored for various lengths of time prior to staining. Due to the time intervals required for cell activation, BrdU incubation and other factors that are necessary to prepare cells prior to BrdU staining, it is often desirable to store samples and complete the staining protocol at a later time. If short term storage of samples prior to staining is desired, staining protocol option no. 1 allows storage of cells overnight after the initial fixation step. If longer storage of samples is desired, staining protocol option no. 2 permits storage of frozen samples indefinitely following the initial fixation step. The flexibility of the BD Pharmingen BrdU Flow Kit staining protocol provides researchers with several time-saving options.

Overview: The BD Pharmingen™ BrdU Flow Kit Staining Protocol



1. Recipe for Freezing Media: 10% dimethyl sulfoxide (DMSO) + 90% heat-inactivated Fetal Bovine Serum (FBS).
2. As an alternative, the staining procedure may be stopped following the DNase treatment step. The DNase-treated cells can then be stored in BD Perm/Wash™ Buffer overnight with staining continued the next day.
3. The immunofluorescent staining of cell surface antigens can be done at the same time as staining intracellular antigens provided that the antibodies recognize paraformaldehyde-fixed epitopes.
4. If staining for total DNA content is not desired, then the 7-AAD staining step can be omitted and fluorescent data for another parameter can then be measured in the FL3 channel.

Kit Components and Storage

Table 1.

Component	Storage condition	No. of vials
Fluorochrome -anti-BrdU Antibody	4°C	1
BD Cytofix /Cytoperm Buffer	4°C	1
BD Perm/Wash Buffer (10×)	4°C	2
BD Cytoperm Plus Buffer	4°C	1
BrdU (10 mg/ml)	-80°C	5
DNase	-80°C	5
7-AAD	4°C	1

Some kit reagents are supplied as concentrated stock solutions and need to be diluted either with deionized H_20, 1× Dulbecco's PBS (DPBS), or with BD Perm/Wash Buffer. Instructions for handling, preparation, and storage of kit components follow:

Fluorochrome-conjugated anti-BrdU Antibody. This vial contains 50 µl stock solution of Fluorochrome-conjugated anti-BrdU antibody that is sufficient for staining 50 samples (10^6 cells/sample). The FITC BrdU Flow Kit (Cat. No. 559619) comes with 50 µl of FITC conjugated anti-BrdU antibody. In the APC BrdU Flow Kit (Cat. No. 552598), 50 µl of APC conjugated anti-BrdU antibody stock is provided. Prior to use, an appropriate amount of the stock antibody solution should be diluted 1:50 with 1× BD Perm/Wash Buffer. 50 µl of the diluted antibody is used to stain each sample. The Fluorochrome anti-BrdU antibody should be stored in the dark at 4°C.

BD Cytofix/Cytoperm™ Buffer. BD Cytofix/Cytoperm Buffer constitutes a single-step fixation and permeabilization reagent that is designed for use in intracellular staining. It contains a mixture of the fixative paraformaldehyde and the detergent saponin. This reagent serves to preserve cell morphology, fix cellular proteins, and permeabilize cells for the subsequent immunofluorescent staining of intracellular proteins. A 25 ml bottle of BD Cytofix/Cytoperm Buffer is provided in a ready to use formulation. BD Cytofix/Cytoperm Buffer should be stored at 4°C.

BD Perm/Wash Buffer. Each 25 ml bottle contains a concentrated (10×) stock solution of BD Perm/Wash Buffer. The BD Perm/Wash Buffer mixture contains fetal bovine serum and the reversible permeabilization detergent reagent, saponin. The concentrated stock buffer should be diluted 1:10 with deionized H_2O; unused portions of 1× BD Perm/Wash Buffer may be stored at 4°C. The two bottles of 10× BD Perm/Wash Buffer that are provided should be stored at 4°C.

Note: The presence of some precipitate in the 10× BD Perm/Wash stock buffer is common. The precipitate will not affect the performance of the buffer. If desired, the precipitate may be removed prior to use by filtration of the diluted 1× BD Perm/Wash Buffer through a 0.45 µm-pore filter.

Note: The BD Perm/Wash Buffer (1×) should be used with fixed cell samples only. Use of this buffer on unfixed cells will cause cell damage.

Note: Source of all serum proteins is from USDA inspected abattoirs located in the United States.

BD Cytoperm™ Plus Buffer. BD Cytoperm Plus Buffer is specially formulated for the BrdU Flow Kit and is used as a staining enhancer and secondary permeabilization reagent (100 µl/sample). One 10 ml bottle of BD Cytoperm Plus Buffer is provided and should be stored at 4°C.

Note: The BD Cytoperm Plus Buffer should be used with fixed cell samples only. Use of this buffer on unfixed cells will cause cell damage.

BrdU. Each vial contains 0.5 ml of a **10 mg/ml** BrdU, (32.5 mM) solution diluted in 1× DPBS. The BrdU solution provided is prepared aseptically, (0.22 µm filtered) and contains no preservative; therefore it is recommended that the solution be handled under aseptic conditions. This stock solution can be injected intraperitoneally (i.p.) into animals or diluted to a 1 mM solution for *in vitro* labeling. For *in vivo* labeling by i.p. injection, proceed to the *in vivo* labeling section of the manual *(**page 11**)*. To label cells *in vitro*, first dilute the stock (10 mg/ml BrdU solution) to a 1mM solution by adding 31 µl to either 1 ml of 1× DPBS or culture medium (this is a dilution of 32×). Add 10 µl of the 1 mM solution to each ml of culture medium to obtain a final concentration of 10 µM. The molecular weight of BrdU is 307.1. Five vials of BrdU Solution are provided and should be stored at –80°C.

Note: The BrdU solution has been shown to be stable for up to 4 months at 4°C or can be refrozen.

DNase. Each vial contains 300 µl of a 1 mg/ml solution of DNase in 1× DPBS. When staining 10 or more samples, thaw the entire vial of DNase solution and add 700 µl of 1× DPBS to make a working stock solution of 300 µg/ml.

Note: If fewer than 10 samples are being treated with DNase, take a 30 µl aliquot of (1 mg/ml) DNase solution/sample and refreeze the remaining 1 mg/ml DNase at –80°C.

Note: DNase stock solution (ie, at 1 mg DNase/ml) may be refrozen once before it loses activity. A total of 100 µl of the working stock is used to treat each cell sample (ie, 30 µg of DNase/ 10^6 cells) with incubations performed at 37°C. Five vials of DNase are provided and should be stored at –80°C.

7-AAD. 7-amino-actinomycin D (7-AAD) is a fluorescent dye for labeling DNA for flow cytometric analysis. A total of 20 µl of 7-AAD is used for staining each sample (10^6 cells/sample). One vial of 7-AAD is provided and should be stored in the dark at 4°C.

Reagents required but not provided

Staining Buffer: 1× DPBS + 3% Fetal Bovine Serum (heat inactivated) + 0.09% sodium azide.

Note: BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) works well for this application (sold separately).

Recipe for 1× DPBS Buffer (1 Liter):

KCl	0.2 g
KH_2PO_4	0.2 g
NaCl	8.0 g
$Na_2HPO_4 \bullet 7H_2O$ (pH 7.2 -7.4)	2.16 g

Warnings and Precautions

BD Cytofix/Cytoperm™ buffer contains 4% paraformaldehyde and is harmful.

- R 40 Limited evidence of a carcinogenic effect
- R43 May cause sensitization by skin contact.
- S 2 Keep out of the reach of children.
- S 13 Keep away from food, drink, and animal feed or feeding materials.
- S 36/37 Wear suitable protective clothing and gloves.
- S 46 If swallowed, seek medical advice immediately and show the container or label.
- S 52 Not recommended for interior use on large surface areas.

The BD Cytoperm™ Plus Buffer contains 10% dimethyl sulfoxide and is harmful.

- R 20/21/22 Harmful by inhalation, if in contact with skin and if swallowed.
- S 25 Avoid contact with eyes.
- S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- S 28 After contact with skin, wash immediately with plenty of water.
- S 36 Wear suitable clothing.
- S 60 This material and its container must be disposed of as hazardous waste.

BrdU contains 1% Broxuridin and is harmful.

- R 20/21/22 Harmful by inhalation, if in contact with skin and if swallowed.
- S 9 Keep container in a well-ventilated place.
- S 28 After contact with skin, wash immediately with plenty of water.
- S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
- S 60 This material and its container must be disposed of as hazardous waste.

Fluorescently conjugated antibodies contain less than 0.1% sodium azide. Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

BrdU Labeling and the BrdU Flow Kit Staining Protocol

Labeling of Cells with BrdU

In vitro-labeling of cultured cells and cell lines with BrdU

Many different protocols for *in vitro* BrdU labeling of cells have been reported.[12-15] We have found that incubating cells with BrdU at a final concentration of 10 µM in cell culture medium (ie, 10 µl of 1 mM BrdU per ml of culture medium) was effective for labeling a wide variety of human and mouse cell lines and normal cell populations.[15, 16] Prolonged exposure of cells to BrdU allows for the identification of actively-cycling cell populations. Pulse labeling of cells by brief BrdU exposures at various time points permits the determination of cell-cycle kinetics.

To label cells *in vitro*, carefully add 10 µl of BrdU solution (1 mM BrdU in 1× DPBS) directly to each ml of tissue culture media. For this step, it is important to avoid disturbing the cells in any way (eg, by centrifugation steps or temperature changes) that may disrupt their normal cell cycling patterns. The cell culture density should not exceed 2×10^6 cells/ml. The treated cells are then incubated for the desired length of time. For pulse-labeling experiments, the choice of time points and lengths of time for pulsing depend on the test cell population's rate of cell cycle entry and progression. For example, an effective length of time for pulsing an actively proliferating cell line (eg, CTLL-2 cells) is 30 – 45 minutes (ie, when the cells are in the logarithmic phase of cell proliferation). Researchers should determine time points and pulse-labeling time intervals that are optimal for each different cell line or cell population within a particular experimental system. Cells from the same population that are not BrdU-labeled are the recommended negative staining control for this assay. This will allow determination of background staining levels for the anti-BrdU monoclonal antibody.

Methods for *in vivo*-labeling of mouse cells with BrdU

Two common methods reported for *in vivo* BrdU labeling of cells include the intraperitoneal (i.p.) injection of a BrdU-containing solution into mice and the feeding of mice with BrdU that is added to their drinking water;[16-22] however, these methods are not routinely tested at BD Biosciences Pharmingen.

Method No. 1: Method for injecting BrdU via the intraperitoneal route. A 10 mg/ml solution of BrdU in sterile 1× DPBS is provided for *in vivo* use. Inject mice i.p. with 100–200 µl (1–2 mg) of BrdU solution.[17, 19, 21] Incorporation of BrdU can be readily detected in thymus and bone marrow in as little as 1 hr post injection.

Method No. 2: Introduction of BrdU through drinking water. Dilute BrdU to 0.8 mg/ml in the drinking water. The BrdU mixture should be made up fresh and changed daily.[18, 23] Prolonged feeding of BrdU can have toxic effects for the animal. Some researchers have reported lethal effects associated with 14 days of continuous BrdU feeding.[21] For longer term studies, some researchers have reported that feeding mice with BrdU for 9 consecutive days followed by a changeover to normal water has worked effectively.[18] BrdU incorporation by cells from these animals has been detected past 70 days.[18]

BrdU Flow Kit Staining Protocol

1. **Immunofluorescent staining of cell surface antigens.**
   a. Add BrdU-pulsed cells (10^6 cells in 50 µl of staining buffer) to flow cytometry tubes.
   b. Add fluorescent antibodies specific for cell-surface markers in 50 µl of staining buffer (eg, BD Pharmingen™ Stain Buffer (FBS) Cat. No. 554656) per tube and mix well.
   c. Incubate cells with antibodies for 15 minutes on ice.
   d. Wash cells 1× by adding 1 ml of staining buffer per tube, centrifuge (5 min.) at 200 – 300 × *g*, and discard supernatant.
2. **Fix and permeabilize cells with BD Cytofix/Cytoperm Buffer.**
   a. Resuspend cells with 100 µl of BD Cytofix/Cytoperm Buffer per tube.
   b. Incubate cells for 15 – 30 minutes at room temperature or on ice.
   c. Wash cells 1× with 1 ml of 1× BD Perm/Wash Buffer, centrifuge as in step 1d and discard supernatant.
3. **Incubate cells with BD Cytoperm Plus Buffer.**
   a. Resuspend cells with 100 µl of BD Cytoperm Plus Buffer per tube.
   b. Incubate cells for 10 minutes on ice.
   c. Wash cells 1× by adding 1 ml of 1× BD Perm/Wash Buffer (as in Step 2c).
4. **Re-Fixation of cells**
   a. Resuspend cells with 100 µl of BD Cytofix/Cytoperm Buffer per tube.
   b. Incubate cells for 5 minutes at room temperature or on ice.
   c. Wash cells 1× by adding 1 ml of 1× BD Perm/Wash Buffer (as in Step 2c).
5. **Treatment of cells with DNase to expose incorporated BrdU.**[24, 25]
   a. Resuspend cells with 100 µl of diluted DNase (diluted to 300 µg/ml in DPBS) per tube, (ie, 30 µg of DNase to each tube).
   b. Incubate cells for 1 hour at 37°C.
   c. Wash cells 1× by adding 1 ml of 1× BD Perm/Wash Buffer (as in Step 2c).
6. **Stain BrdU and intracellular antigens with fluorescent antibodies.**
   a. Resuspend cells with 50 µl of BD Perm/Wash Buffer containing diluted fluorescent anti-BrdU and/or antibodies specific for intracellular antigens.
   b. Incubate cells for 20 minutes at room temperature.
   c. Wash cells 1× by adding 1 ml of 1× BD Perm/Wash Buffer (as in Step 2c).
7. **Optional — Staining of total DNA for cell cycle analysis.**

   Note: Proceed to Step 8 if the staining of total DNA levels is not desired.

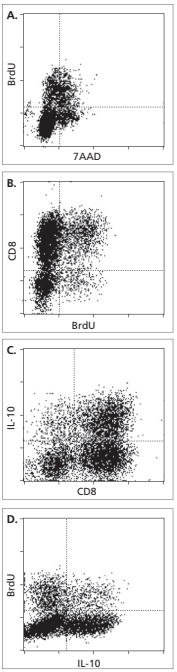
   a. Resuspend cells with 20 µl of the 7-AAD solution.
8. **Resuspension of cells for Flow Cytometric Analysis.**
   a. Add 1 ml of staining buffer to each tube to resuspend cells.
   b. Analyze stained cells with a flow cytometer (run at a rate no greater than 400 events/sec.) and acquire multiparameter data files.

   Note: Samples may be stored overnight at 4°C, protected from exposure to light, prior to analysis by flow cytometry.

Flow Cytometric Analysis of Stained Cell Samples

Some of the flow cytometric data presented in the following examples was acquired using a flow cytometer equipped with a 488 nm argon laser. This laser permits the excitation of the fluorescent dyes, fluorescein isothiocyanate (FITC) (FL1), phycoerythrin (PE) (FL2), and 7-AAD (FL3), as well as the generation of forward angle (FSC) and side-scattered (SSC) light signals from illuminated cells. When staining with fluorochromes which are excited by light wavelengths outside the range generated by the argon laser, such as allophycocyanin (APC), flow cytometers such as the BD FACSCalibur™ that have an additional laser light source were used. It should be noted that with the addition of each different fluorochrome used for multicolor staining, it becomes more critical to properly compensate overlaps in detection of emitted fluorescent signals. Fluorescent signals from the DNA-content marker 7-AAD are normally acquired in the linear signal amplification mode, whereas fluorescent signals generated by other fluorochromes are typically acquired in a logarithmic mode.

Sample Data using the BD Pharmingen FITC BrdU Flow Kit Protocol



Panel A: 7-AAD versus FITC anti-BrdU. From this plot, one can determine the percentage of cells that have incorporated BrdU or the percentage of cells in the S (DNA synthesis) phase of the cell cycle. Applying quadrant markers to the flow cytometric data revealed that 27% of the cells actively incorporated BrdU.

Panel B: APC anti-CD8 versus FITC anti-BrdU. From the data, the frequency of $CD8^+$ cells that incorporated BrdU was 24%.

Panel C: APC anti-CD8 versus PE anti-IL-10. From the data, the frequency of $CD8^+$ IL-10-producing cells was calculated. It was determined that 34% of the $CD8^+$ cells produced IL-10.

Panel D: PE anti-IL-10 versus FITC anti-BrdU. From the data, the frequency of IL-10-producing cells that incorporated BrdU was 38%.

Figure 1. Multicolor flow cytometric analysis of stimulated mouse cells that synthesized DNA and/or produced IL-10. Spleen cells from a BALB/c mouse were primed *in vitro* and restimulated with PMA and ionomycin in the presence of a protein transport inhibitor (to promote intracellular cytokine accumulation). During the final 45 minutes of culture, the cells were labeled with 10 µM BrdU. The cells were then harvested and stained with FITC anti-BrdU (FL1), PE anti-IL-10 (FL2), 7-AAD (FL3), and APC anti-CD8 (FL4). The panels depict two-color staining patterns generated from the reanalyzed flow cytometric data for these cells. A great deal of information can be obtained for a given cell population stained using this multiparameter staining technique. ***(see panel descriptions)***

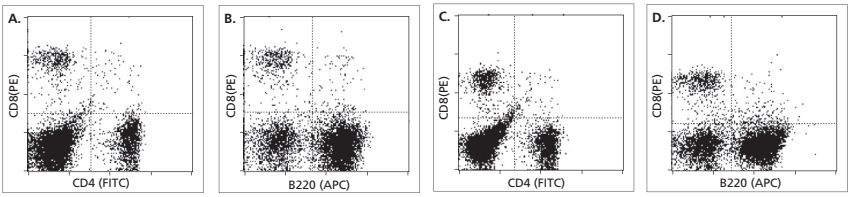


Figure 2. Analysis of cell surface antigen expression using the BrdU Flow Kit. Comparison with a conventional cell surface antigen-staining method. Mouse splenocytes were stained (with FITC anti-CD4, PE anti-CD8, and APC anti-B220) and fixed with BD Cytofix/Cytoperm™ Buffer ***(Panels A & B)***. Similar samples were treated according to the BD Pharmingen BrdU staining protocol ***(Panels C & D)***. Two-color staining patterns generated from the reanalyzed flow cytometric data for these cells are shown in Panels A & C: CD4 (FITC) versus CD8 (PE) and Panels B & D: B220 (APC) versus CD8 (PE). The percentages of cells that expressed CD4, CD8, or B220 and their Mean Fluorescence Intensities (MFI) are summarized in Table 2.

Table 2. Comparison of the BrdU and conventional staining protocols

	% Positive		MFI	
	Conventional Protocol	**BrdU Protocol**	**Conventional Protocol**	**BrdU Protocol**
CD4+ (FITC)	20.5	21.5	278	141
CD8+ (PE)	8.4	9.0	649	195
B220+ (APC)	62.9	60.1	261	189

Table 2. Summary of Figure 2. Comparison of the BrdU and conventional staining protocols for the detection of cell surface antigen expression. While there was a decrease in signal intensity for all markers stained using the BrdU staining protocol, there were no observed differences in ability to distinguish the stained cell populations ***(as shown in Table 2)***. The decrease in signal intensity for samples stained using the BrdU staining protocol did not adversely affect the percentage of cells stained for these markers, however, the detection of surface antigens expressed in low levels may be affected by staining with the BrdU technique.

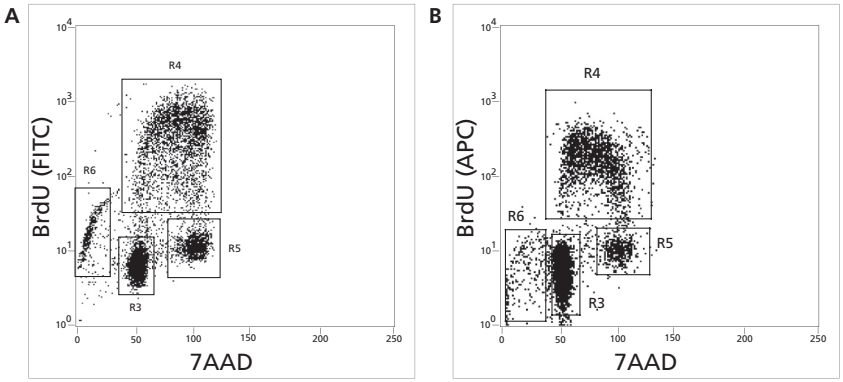


Figure 3. Region gates for the quantitative cell cycle analysis of populations that have been stained for incorporated BrdU and total DNA levels. Panel A: The measurement of cell incorporated BrdU (with FITC anti-BrdU) and total DNA content (with 7-AAD) in D10.G4.1 cells. The D10.G4.1 cells were cultured with 10 µM BrdU for 30 minutes. The cell cycle positions and active DNA synthetic activities of cells can be determined by analyzing the correlated expression of total DNA and incorporated BrdU levels. As shown by the region gates applied to the 7-AAD versus BrdU dot plot, flow cytometric analysis of cells stained with the reagents provided in the BrdU Flow Kit allowed for the discrimination of cell subsets that were apoptotic (defined as sub-G0/G1, R6, 5.6% of cells) or resided in G0/G1 (R3, 38.6%), S (R4, 38.6%), or G2 + M (R5, 14.4%) phases of the cell cycle and had recently synthesized DNA.5, 6 The 7-AAD signal data was acquired in a linear mode as shown by the x-axis scale. ***Panel B:*** The measurement of cells-incorporated BrdU (with APC anti-BrdU) and total DNA content (with 7-AAD). Human PBMCs were stimulated with immobilized anti-human CD3 antibody, clone HIT3a, 10ug/ml for plate coating, (Cat. No. 555336), soluble anti-human CD28 antibody, clone CD28.2 at 2ug/ml, (Cat. No. 555725), recombinant human IL-2 at 10ng/ml (Cat. No. 554603) and recombinant human IL-4 at 20ng/ml (Cat. No. 554605) for 2 days. The cells were then washed and subsequently expanded for 3 days in culture with medium containing recombinant IL-2 and IL-4. Finally the cells were harvested and restimulated for 4 hours with PMA (Sigma, Cat. No P-8139 5ng/ml) and ionomycin (Sigma, Cat. No. I-0634 500ng/ml). 20uM BrdU was added for the final hour. Region gates are as in figure 3A with region 6 being apoptotic (3.31%), region 4 is S phase(23.5%), region 3 is G0/G1(64.3%), and region 5 is G2+M (6.1%).

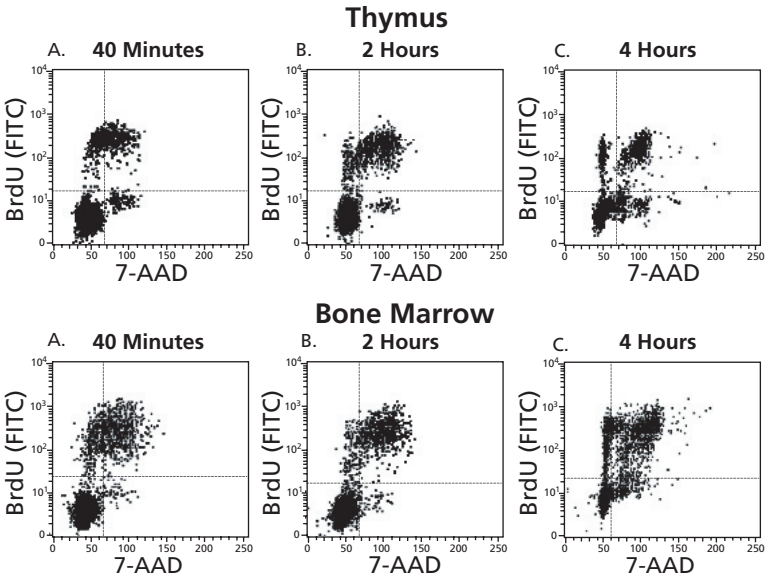
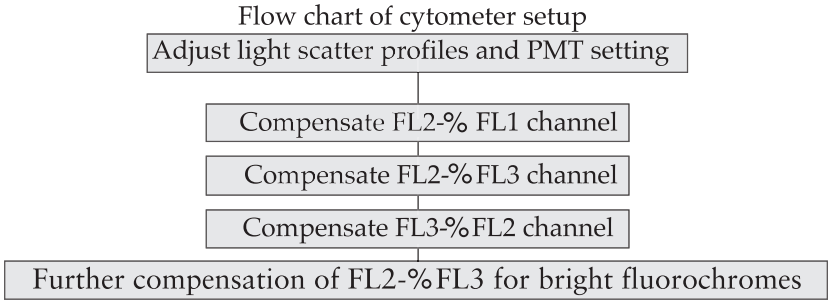


Figure 4. Time course study of in vivo BrdU pulsing in mice. C57BL/6 mice were intraperitoneally injected with 1 mg of BrdU for various time intervals. The animals were sacrificed at 40 minutes, 2 hours or 4 hours post injection. Thymus and bone marrow cells were removed and then stained for BrdU and 7AAD. Panel A shows bone marrow and thymus cells obtained from mice that were pulsed with BrdU for 40 minutes. Notice the characteristic BrdU/7AAD "horseshoe" fluorescence staining profile. Panel B shows staining patterns for bone marrow and thymus cells from mice pulsed for 2 hours. The characteristic horseshoe pattern is present. In addition, a population of cells that have incorporated BrdU but reside in the G0-G1 compartment is discernible (ie, BrdU-positive cells without increased 7AAD content). Panel C shows bone marrow and thymus cells from mice pulsed for 4 hours. The profile now shows a large population of BrdU-positive cells that are in G0-G1. The characteristic BrdU/7AAD horseshoe pattern is much less discernible.

Flow Cytometer Setup Guidelines



Instrument setup and adjustment of compensation values is a complex procedure. The information in ***Figures 5 – 15*** is intended as an example of the type of instrument setup necessary for samples stained using the BrdU staining procedure. ***Figures 5 – 10*** are specific to the BD Pharmingen™ FITC BrdU Flow Kit (Cat. No. 559619) and ***Figures 11 – 15*** refer to the BD Pharmingen™ APC BrdU Flow Kit (Cat. No. 552598). In addition, the instrument adjustments required might vary between instruments and between individual samples in a given experiment. It is often necessary to make further adjustments for different combinations of fluorescent-conjugated antibodies. It is recommended that the researcher refer to a textbook on flow cytometry or on cell-cycle analysis by flow cytometry for more information.[26, 27]

Note: Refer to Howard M. Shapiro's textbook, *Practical Flow Cytometry* (3rd Edition, Wiley-Liss, New York) for additional information on flow cytometers and flow cytometry.

FITC BrdU Instrument Set up

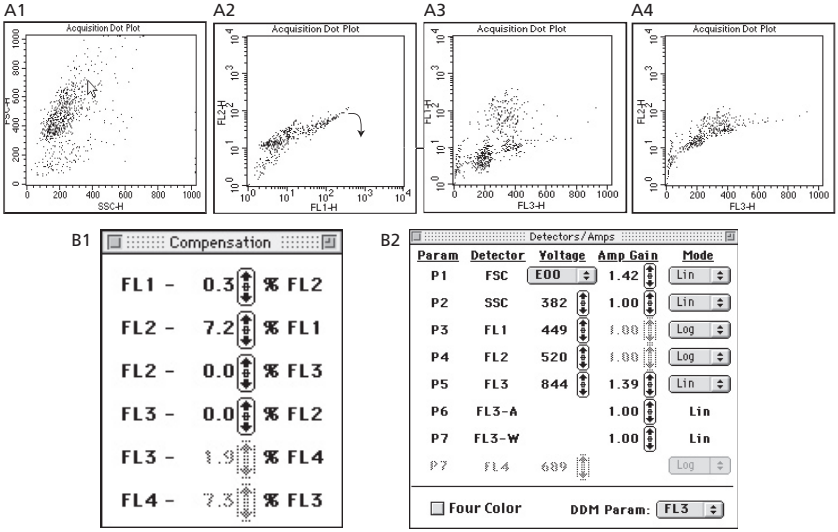


Figure 5. Initial instrument settings for analysis using the FITC BrdU Flow Kit. Mouse T-cells were primed in vitro and reactivated with PMA and ionomycin in the presence of a protein transport inhibitor. The cells were pulse-labeled with 10 µM BrdU for the final 45 minutes of stimulation. The cells were harvested, fixed, permeabilized, re-fixed and treated with DNase using the BrdU Flow Kit protocol. The samples were stained with FITC anti-BrdU (FL1), 7-AAD (FL3), and either PE Immunoglobulin (Ig) Isotype Control (FL2), PE anti-mouse IL-10 or PE anti-mouse TNF. The sample was analyzed by flow cytometry as shown in ***Panels 5.A1 - A4***. Initial flow cytometer PMT voltage and fluorescence compensation settings were generated using the BD FACSComp™ Beads and Software in Lyse/Wash mode ***(Panels 5.B1-B2)***. Using the BD CellQuest™ flow cytometry analysis software, bivariate dot plot windows were created for acquisition and subsequent analysis of data as shown in ***Panels 5.A1 - A4***. The PMT voltage settings ***(Panel 5.B2)*** were specified to generate a typical SSC versus FSC light scatter plot ***(Panel 5.A1)*** for mouse lymphocytes. The FL3 detector was set to Linear Mode ***(Panel 5.B2)*** for the acquisition and storage of data generated for cells stained for DNA content using 7-AAD. The intensity of 7-AAD signal (FL3) was adjusted by changing the FL3 PMT voltage setting such that the mean fluorescence intensity (MFI) of the G0/G1 population ***(see Figure 3)*** is = 50 (for 256 linear resolution) or 200 (for 1024 linear resolution) as shown in ***Panel 5.A3***. The FL1 and FL2 detectors were set to Logarithmic Mode for acquisition and storage of fluorochrome emission by immunofluorescently stained cells. The baseline PMT voltages were adjusted so that the mean fluorescence intensity of the unstained cell population (autofluorescence) fell within the first decade ($\leq 10^1$) of the fluorescence intensity scale ***(data not shown)***.

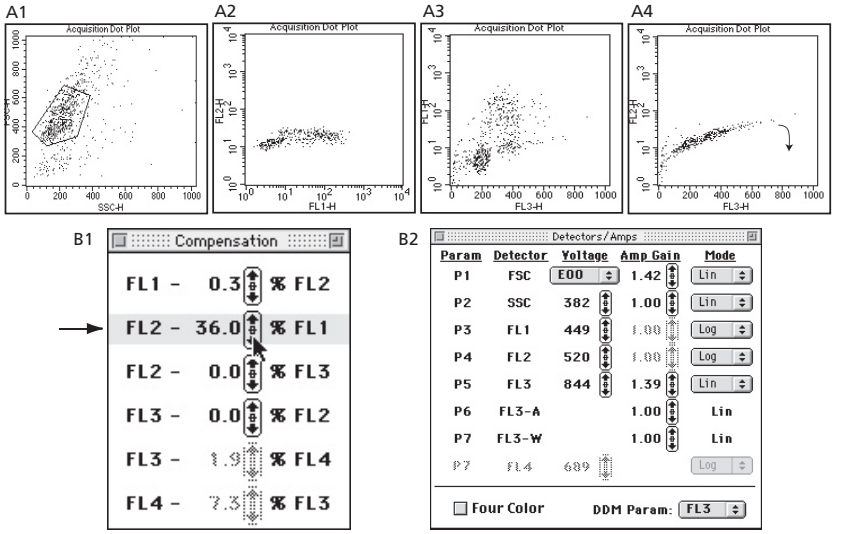


Figure 6. Compensation of FITC (FL1) emission. In this figure a region gate was drawn around the cell population of interest (in this case lymphocytes) based on their light scattering characteristics ***(Panel 6.A1)*** and that gate was applied to the other analysis plots ***(Panels 6.A2 - A4)***. This figure also shows results from samples ***(as described in Figure 5)*** after adjustment of the FL2 – %FL1 compensation setting to eliminate the spectral overlap contributed by FITC anti-BrdU+ cells (FL1). The increase in FL2 – %FL1 compensation aligned the individual cell mean FL2 fluorescence intensities so that the entire cell population was parallel with respect to the x-axis as shown in ***Panel 6.A2***.

Note: The PE (FL2) emission of this sample was low due to staining with a PE Ig isotype control.

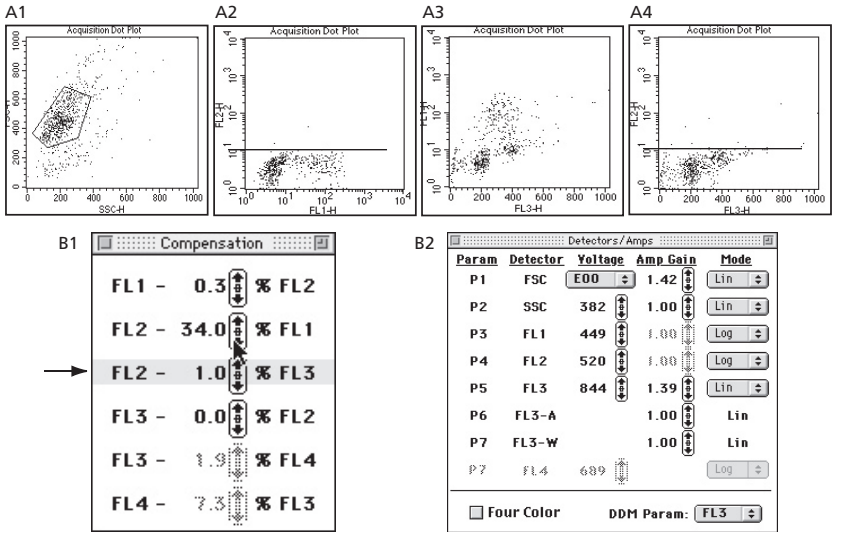


Figure 7. Compensation of 7-AAD (FL3) emission. This figure shows results from samples ***(as described in Figure 5)*** after adjustment of the FL2 – %FL3 compensation setting to eliminate the spectral overlap contributed by 7-AAD+ cells (FL3). This adjustment brought the FL2 intensity of the cell population within the first decade (MFI ≤ 10) as shown in ***Panel 7.A2***. The FL2 – %FL3 compensation setting was very sensitive and only required minor changes.

Note: The PE (FL2) emission of this sample was low due to staining with a PE Ig isotype control.

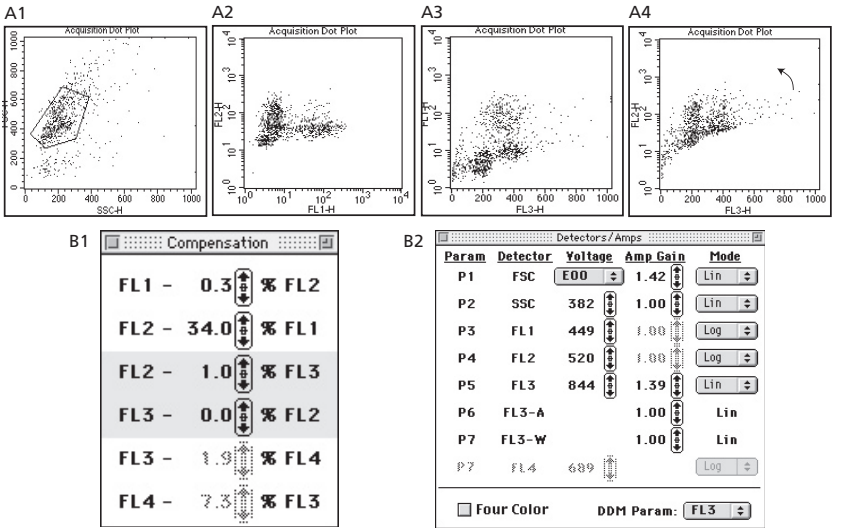


Figure 8. Effects caused by the undercompensation of PE (FL2) emission. This figure shows immunofluorescent staining with PE anti-IL-10, FITC anti-BrdU and 7-AAD (cells were stained as described in ***Figure 5)***. The PMT voltages and compensation settings established for the PE-Ig isotype control were found to be inadequate for compensating cytokine-producing cells that were stained with PE anti-IL-10 ***(Panels 8.A1 - A4)***.

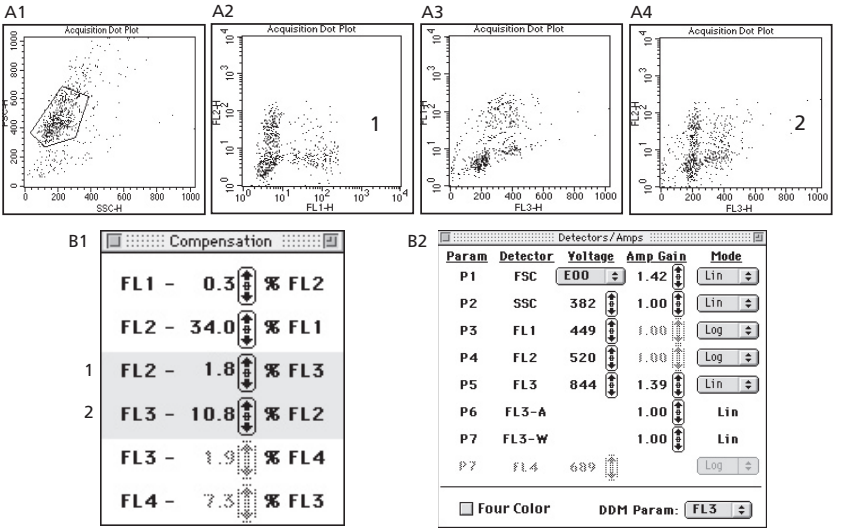


Figure 9. Corrected compensation for PE (FL2) emission. This figure depicts the cells from ***Figure 8*** that were stained with PE anti-mouse IL-10 after compensation of FL2 – %FL3 (1) and FL3 – %FL2 ***(2)***. The increased FL2 – %FL3 compensation setting eliminated the spectral overlap contributed by 7-AAD (FL3) and as a result caused a reduction in the mean FL2 fluorescence intensity of the entire cell population ***(1)***. This adjustment brought the FL2 intensity of the IL-10- cell population within the first decade (MFI ≤ 10). The FL3 – %FL2 compensation setting was also increased ***(see Figure 8.B1 and Panel 9.B1)*** to eliminate spectral overlap contributed by PE anti-IL-10 (FL2) as seen in ***Panel 8.A4 (2)***.

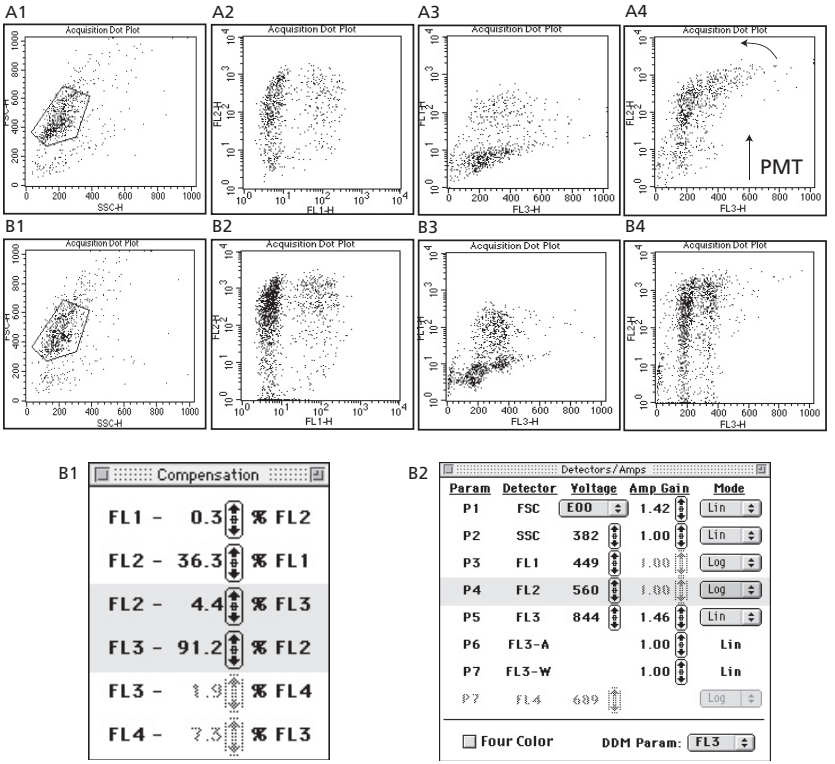


Figure 10. Compensation necessary for differences in PE (FL2) emission. This figure depicts the cells described in Figure 5 that were stained with PE anti-TNF in addition to FITC anti-BrdU and 7-AAD. ***Panels 10.A2 - A4*** show the staining of PE anti-TNF antibody when analyzed using the settings established for samples stained with PE anti-IL-10 ***(see Figure 9)***. The FL2 signal intensity of the TNF+ population was much greater than the signal intensity of the population stained with PE anti-mouse IL-10 ***(Figure 9.A2 and Panel 10.A2)***. These data emphasize the need to adjust compensation settings due to changes in fluorescence emission intensities between samples stained with different fluorochrome conjugated antibodies. The increased PE (FL2) signal intensity resulted in a loss of the characteristic cell population staining profiles - as shown in A3, due to enhanced spectral overlap of the TNF+ cells ***(Panels 10.A2 - A4)***. Since the PE (FL2) emission of this sample was collected in Log Mode and the 7-AAD (FL3) emission in Linear Mode, the increased spectral overlap associated with the TNF+ cells could not effectively be eliminated solely by adjustments made to the compensation settings. Compensation of the spectral overlap associated with this sample required two types of adjustments. First, the FL2 PMT voltage setting was increased to allow for effective compensation of FL2 spectral overlap. Subsequently similar adjustments were made to the FL3 – %FL2 and to the FL2 – %FL3 settings as was described in ***Figures 7 - 9***. ***Panels 10.B2 - B4*** show data acquired with the corrected instrument settings.

Note: Additional fine adjustments to the FL2 – %FL1 and FL1 – %FL2 compensation settings may be required after changes in PMT voltages.

APC BrdU Instrument Set up

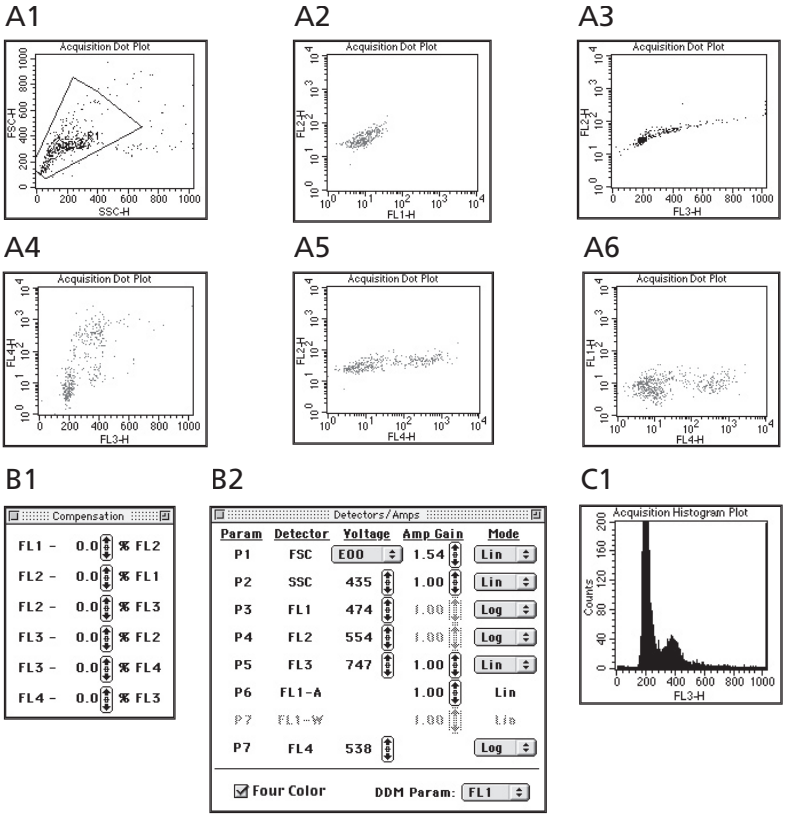


Figure 11. Initial instrument settings. When staining cells with anti-BrdU APC the initial set up is very similar to the FITC anti-BrdU conjugate shown in ***Figure 5***. Shown here are cells stained with isotype controls for FITC and PE, 7-AAD and APC anti-BrdU. The important difference here is that APC anti-BrdU is detected with a separate laser. ***Figure 11.A1*** shows a typical SSC vs. FSC light scatter plot with a gate around the cell population of interest. Note the FL3 detector is set to the linear mode for the acquisition and storage of data generated for cells stained for DNA content using 7-AAD. The adjustments for FL3 are described in detail in ***Figure 7*** and shown here in ***Figure11.B2***. Increase the FL4 voltage to allow for the BrdU- population to fall within the first decade of the fluorescent intensity scale as shown in 11.A4 and A.6. Increase the FL3 PMT (7AAD histogram) to set the G0/G1 population on the 50 if using the 256 linear scale or 200 for the 1024 scale as shown in ***Figure 11.C1 and 11.A4***.

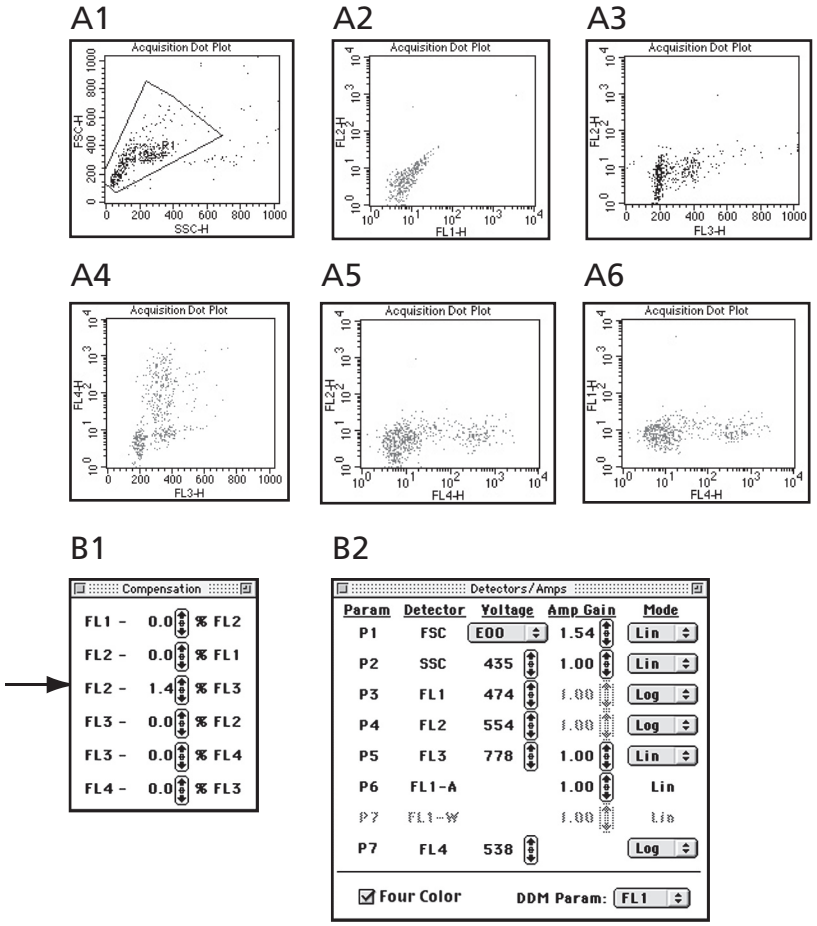


Figure 12. Compensation of PE conjugates. Once you have set the FL3 and FL4 PMT voltages, proceed to adjusting the compensation. To begin with there may be a slight bleed over of signal from the PE conjugates, measured in FL-2, into the FL-4 channel. There is no direct compensation for FL4-%FL2. To adjust for this we suggest using the FL-2-%FL3. This compensation tool allows a decrease of the FL2 signal without decreasing the voltage. Here again we are using the FITC and PE isotype controls. By increasing the FL2-%FL3 compensation, in this example we increase to 1.4, the spectral overlap contributed by 7-AAD is eliminated as seen by comparing FL2 in ***Figures 11.A2, .A3, .A5 and 12.A2, .A3, and .A5.***

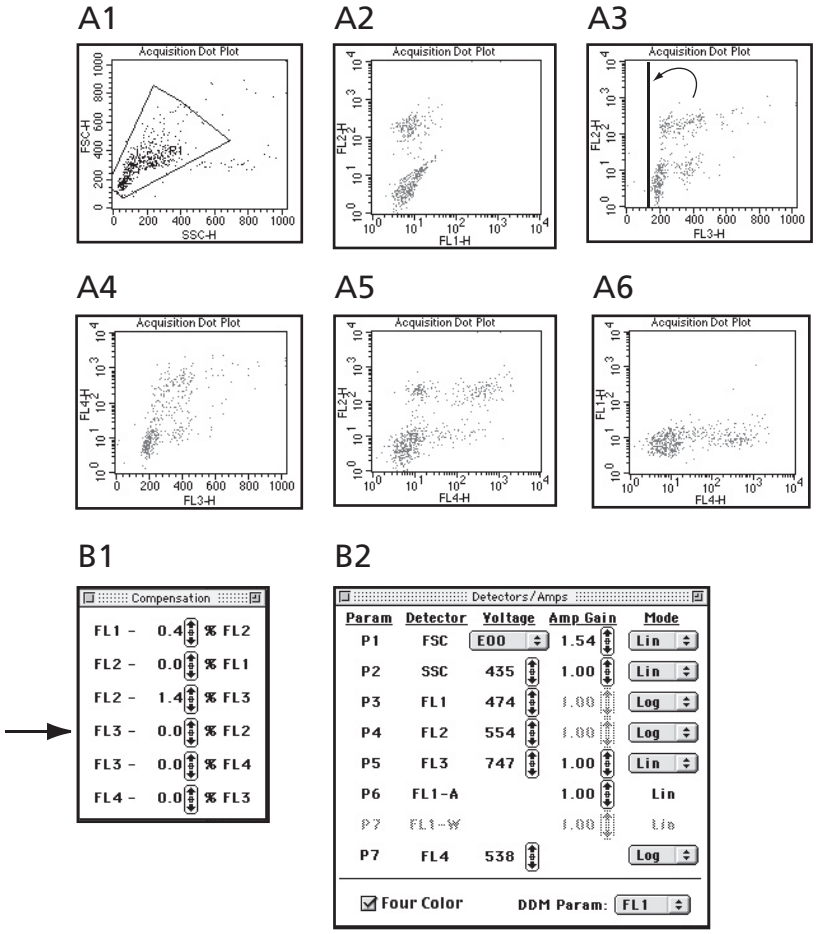


Figure 13. Identification of undercompensation when looking at a PE (FL2) positive population. The compensation between FL-2 and FL-3 will need to be adjusted when analyzing a PE positive cell population. This figure shows cells stained positively with PE anti-Ter-119 (Cat. No. 553673) as well as 7AAD, APC anti-BrdU and an FITC isotype control. To eliminate the spectral overlap incurred when cells are stained brightly with PE it is necessary to adjust FL3-%FL2 so that the PE positive population is directly over the negative population, parallel to the Y-axis. This is necessary to maintain the characteristic BrdU/7AAD horseshoe profile. The results of these adjustments are shown in ***Figure 14***.

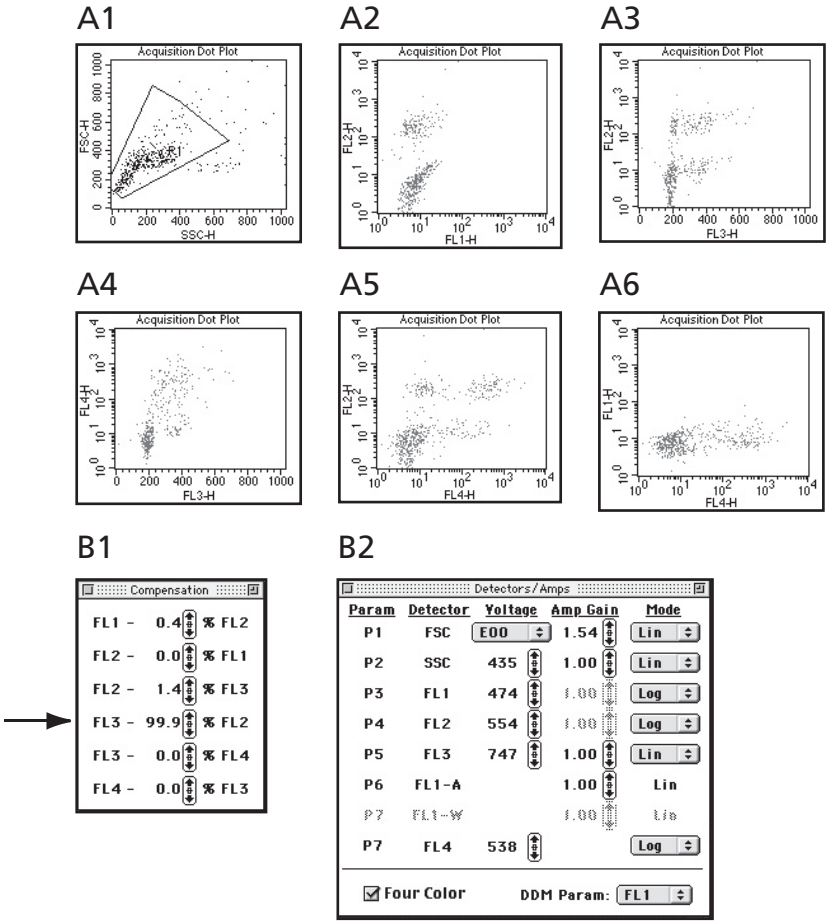


Figure 14. Corrected compensation for PE (FL2) emission. By adjusting the FL3-%FL2 compensation the PE positive population is now directly aligned over the PE negative population as seen in ***14.A3 vs. 13.A3***. As a result, the G0/G1, S and G2/M populations can clearly be seen in the APC anti-BrdU, 7AAD plot, FL-3 vs. FL-4, ***Figure 14.A4***.

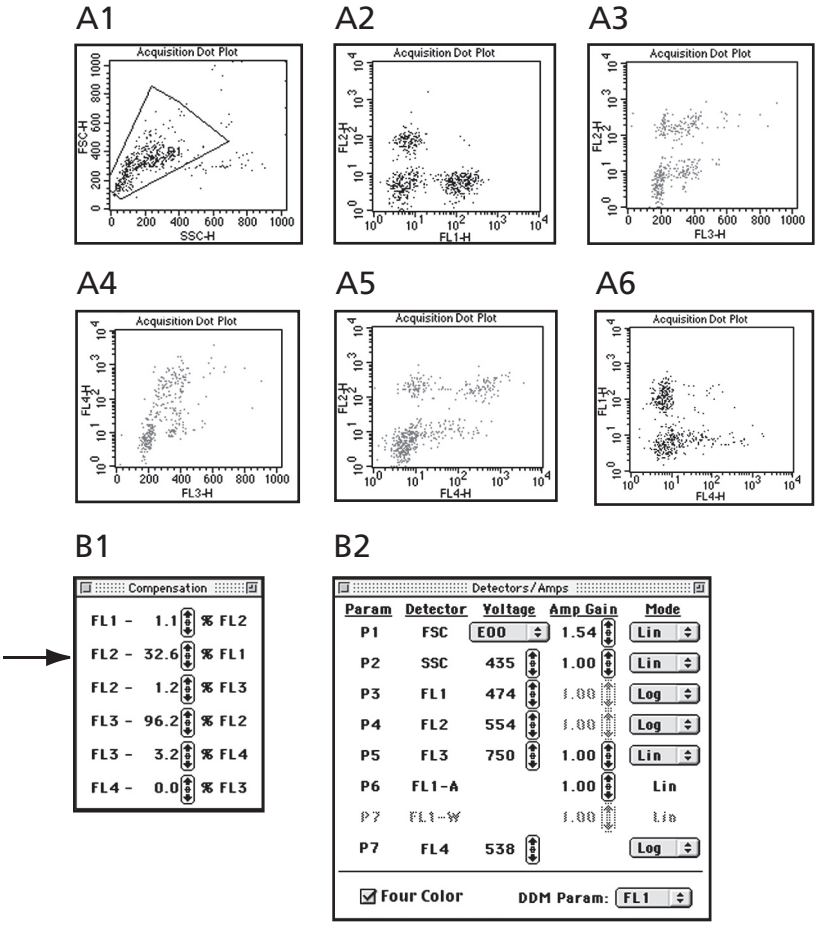


Figure 15. Corrected compensation for FITC (FL2) emission. In this figure the cells have been stained with FITC anti-CD11b (Cat. No. 557396), as well as PE anti-Ter-119 (Cat. No. 553673), 7AAD and APC anti-BrdU. Here the FITC overlap must be removed from the FL2 channel. By adjusting the FL2-%FL1 compensation the PE positive population will be aligned over the PE negative population This compensation is basic FITC vs PE compensation. No other adjustments are needed. All of the panels are now compensated and acquisition may now occur. Since different PE antibodies are brighter then others, further adjustments of the FL2-%FL3 and FL3-%FL2 may still be necessary during the experiment. Also since the FL3 signal of the 7AAD is run in the linear mode some fine adjustments to maintain the G0/G1 profile on either the "50" or "200" may be needed during the experiment. When increases or decreased in the FL3 PMT are done the compensation of the FL2-%FL3 may need to be adjusted. Please refer to the FITC compensation section of this manual for further PE vs 7AAD compensation instructions.

Staining and Analysis Tips

Choice of Fluorochrome

While the BD Pharmingen BrdU Flow Kit staining technique is more compatible with multicolor immunofluorescent staining than most BrdU and intracellular staining protocols, the immunofluorescent staining patterns for cell surface antigens may still be affected due to differences in fluorescent signal intensities. Fluorescent signals generated by cells labeled with PE-conjugated antibodies are affected the most in this system whereas fluorescent signals generated by APC-conjugated antibodies are least affected. To avoid or minimize this effect, use fluorescent antibodies tagged with a fluorochrome that generates the brightest signal when staining markers that are expressed at low levels.

Choice of Antibody Clone or Reagents

The BD Pharmingen BrdU Flow Kit staining procedure utilizes the fixative paraformaldehyde. The use of paraformaldehyde for fixation can alter epitopes on antigens and inhibit recognition by some antibodies after fixation. It is important that the antibody reagents used to stain proteins with this procedure be capable of binding to paraformaldehyde-fixed epitopes. Reagents that are compatible with other fixatives (eg, ethanol) may not work with the BD Pharmingen BrdU Flow Kit staining procedure. A list of reagents specific for various intracellular antigens that are compatible with the BD Pharmingen™ BrdU Flow Kit staining procedure is included in the Appendix.

Doublet Discrimination and Four Color Analysis With a BD FACSCalibur Instrument:

The BD FACSCalibur™ flow cytometer is capable of four-color analysis by the excitation and measurement of fluorescent emissions from four different fluorochromes (ie, fluorescent antibodies or nucleic acid dyes) using 4 parameters (FL1, FL2, FL3 and FL4). Acquiring fluorescent data on three channels allows for use of the doublet discrimination feature. The use of all four parameters for the detection of dye/fluorescent antibody staining will prohibit the user from using the doublet discrimination feature.

Analysis of BrdU in Tissue Sections

The BD Pharmingen™ BrdU *In-Situ* Detection Kits (Cat. Nos. 551321, 550803) are designed for immunohistochemical staining of BrdU in frozen sections, formalin-fixed paraffin-embedded sections, and cultured or isolated cells on slides. Our monoclonal antibody against BrdU provides improved specific staining with minimal background. The direct biotinylation of the mouse antibody against BrdU works in all species including mouse tissues by eliminating the need for a species-specific secondary antibody. The BD Pharmingen™ BrdU *In-Situ* Detection Kit features our BD Retrievagen A antigen retrieval solution designed specifically to unmask antigenic sites, preserve tissue morphology, and enable simultaneous staining of other surface antigens in conjunction with BrdU. This important feature of the kit enables study of the proliferation state of phenotypically defined cells within the micro-environment of tissues. Consistent results are assured by providing all critical reagents in addition to a comprehensive series of protocols for staining BrdU in paraffin-embedded and frozen tissue sections and cultured cells, and in conjunction with other antigens. Control slides are provided in the kit to serve as reference.

Appendix

Reagents for Intracellular Cytokine Flow Cytometry

Fluorochrome-Conjugated Anti-Cytokine/Chemokine Antibodies

Note: PE-Labeled Anti-Human Cytokine and Chemokine Antibodies are available in a 100 Test Size for use in Intracellular Cytokine Flow Cytometry. Pre-titered for optimal immunofluorescent staining of fixed and permeabilized cells (20 µl/test), this new format can help assure consistent staining results and reduce assay development time.

Description	Clone	Isotype	Format	Size	Cat. No.
Human Cytokines and Chemokines					
IL-1α	364-3B3-14	Mouse IgG_1, κ	PE	0.1 mg	554561
IL-2	MQ1-17H12	Rat IgG_{2a}	FITC	0.1 mg	554565
			PE	0.1 mg	554566
			PE	100 Tests	559334
			APC	0.1 mg	551383
IL-3	BVD3-1F9	Rat IgG_1	PE	0.1 mg	554676
IL-4	MP4-25D2	Rat IgG_1	FITC	0.1 mg	554484
			PE	0.1 mg	554485
			APC	0.1 mg	554486
			Alexa Fluor® 488	100 Tests	557727
			Alexa Fluor® 647	100 Tests	557738
IL-4	8D4-8	Mouse IgG_1	PE	0.1 mg	554516
			PE	100 Tests	559333
IL-5	TRFK5	Rat IgG_1	PE	0.1 mg	554395
			APC	0.1 mg	554396
IL-5	JES1-39D10	Rat IgG_{2a}	PE	0.1 mg	554489
			PE	100 Tests	559332
IL-6	MQ2-13A5	Rat IgG_1	FITC	0.1 mg	554544
			PE	0.1 mg	554545
IL-6	MQ2-6A3	Rat IgG_{2a}	FITC	0.1 mg	554696
			PE	0.1 mg	554697
			PE	100 Tests	559331
GM-CSF	BVD2-21C11	Rat IgG_{2a}	PE	0.1 mg	554507
GRO	10G4.1	Mouse IgG_1, κ	PE	0.1 mg	555042
IFN-γ	B27	Mouse IgG_1	FITC	0.1 mg	554700
			PE	0.1 mg	554701
			PE	100 Tests	559327
			APC	0.1 mg	554702
			Alexa Fluor® 488	100 Tests	557718
			Alexa Fluor® 647	100 Tests	557729
IFN-γ	4S.B3	Mouse IgG_1, κ	FITC	0.1 mg	554551
			PE	0.1 mg	554552
			PE	100 Tests	559326
IL-8	G265-8	Mouse IgG_{2b}	FITC	0.1 mg	554719
			PE	0.1 mg	554720
IL-10	JES3-9D7	Rat IgG_1	PE	0.1 mg	554498
			PE	100 Tests	559337

Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, Or.

Description	Clone	Isotype	Format	Size	Cat. No.
Human Cytokines and Chemokines *(Continued)*					
IL-10	JES3-19F1	Rat IgG_{2a}	PE	0.1 mg	554706
			PE	100 Tests	559330
			APC	0.1 mg	554707
IL-12 (p40/p70)	C11.5	Mouse IgG_1	FITC	0.1 mg	554574
			PE	0.1 mg	554575
			PE	100 Tests	559329
			APC	0.1 mg	554576
IL-12 (p70)	20C2	Rat IgG_1, κ	PE	0.1 mg	557020
			PE	100 Tests	559325
IL-13	JES10-5A2	Rat IgG_1	PE	0.1 mg	554571
			PE	100 Tests	559328
IL-16	14.1	Mouse IgG_{2a}, κ	PE	0.1 mg	554736
IP-10	6D4/D6/G2	Mouse IgG_{2a}, κ	PE	0.1 mg	555049
MCP-1	5D3-F7	Mouse IgG_1	PE	0.1 mg	554666
			PE	100 Tests	559324
MCP-3	9H11	Mouse IgG_1	PE	0.1 mg	555033
MIG	B8-11	Mouse IgG_1, κ	PE	0.1 mg	555039
MIP-1α	11A3	Mouse IgG_{2a}, κ	PE	0.1 mg	554730
RANTES	2D5	Mouse IgG_1	PE	0.1 mg	554732
			PE	100 Tests	559322
Thioredoxin	2G11/TRX	Mouse IgG_1	FITC	0.1 mg	559968
TNF	MAb11	Mouse IgG_1	FITC	0.1 mg	554512
			PE	0.1 mg	554513
			PE	100 Tests	559321
			APC	0.1 mg	554514
LT-α (TNF-β)	359-81-11	Mouse IgG_1	PE	0.1 mg	554556
Mouse Cytokines and Chemokines					
IL-1α	ALF-161	Hamster IgG	PE	0.1 mg	559810
IL-2	JES6-5H4	Rat IgG_{2b}	FITC	0.1 mg	554427
			PE	0.1 mg	554428
			APC	0.1 mg	554429
IL-3	MP2-8F8	Rat IgG_1	PE	0.1 mg	554383
IL-4	BVD4-1D11	Rat IgG_{2b}	PE	0.1 mg	554389
IL-4	11B11	Rat IgG_1	PE	0.1 mg	554435
			APC	0.1 mg	554436
IL-5	TRFK5	Rat IgG_1	PE	0.1 mg	554395
			APC	0.1 mg	554396
IL-6	MP5-20F3	Rat IgG_1	PE	0.1 mg	554401
IL-10	JES5-16E3	Rat IgG_{2b}	FITC	0.1 mg	554466
			PE	0.1 mg	554467
			APC	0.1 mg	554468
IL-12 (p40/p70)	C15.6	Rat IgG_1	PE	0.1 mg	554479
			APC	0.1 mg	554480
IL-17	TC11-18H10	Rat IgG_1, κ	PE	0.1 mg	559502
GM-CSF	MP1-22E9	Rat IgG_{2a}	PE	0.1 mg	554406
IFN-γ	XMG1.2	Rat IgG_1	FITC	0.1 mg	554411
			PE	0.1 mg	554412
			APC	0.1 mg	554413
MCP-1	2H5	Hamster IgG, κ	PE	0.1 mg	554443

Description	Clone	Isotype	Format	Size	Cat. No.
Mouse Cytokines and Chemokines *(Continued)*					
TNF	MP6-XT22	Rat IgG_1	FITC	0.1 mg	554418
			PE	0.1 mg	554419
			APC	0.1 mg	554420
TNF	TN3-19.12	Hamster IgG	PE	0.1 mg	559503
Rat Cytokines and Chemokines					
IL-4	OX-81	Mouse IgG_1, κ	PE	0.1 mg	555082
IL-10	A5-4	Mouse IgG_{2b}	PE	0.1 mg	555088
IFN-γ	DB-1	Mouse IgG_1, κ	FITC	100 Tests	559498
			PE	100 Tests	559499
GM-CSF	B61-5	Mouse IgG_1	PE	0.1 mg	555092
MCP-1	2H5	Hamster IgG, κ	PE	0.1 mg	554443
TNF	TN3-19.12	Hamster IgG	PE	0.1 mg	559503
Pig Cytokines and Chemokines					
IFN-γ	P2G10	Mouse IgG_1	PE	0.1 mg	559812

Recombinant proteins useful as specificity controls for intracellular cytokine flow cytometry

Description	Format	Size	Cat. No.
Human			
IL-2	Standard	10 µg	554603
IL-3	Standard	10 µg	554604
IL-4	Standard	5 µg	554605
IL-5	Standard	5 µg	554606
IL-6	Standard	10 µg	550071
IL-8	Standard	20 µg	554609
IL-10	Standard	5 µg	554611
IL-16	Standard	5 µg	554637
GM-CSF	Standard	10 µg	550068
MIP-1α	Standard	10 µg	554622
TNF	Standard	10 µg	554618
LT-α (TNF-β)	Standard	10 µg	554619
Mouse			
IL-2	Standard	20 µg	550069
IL-3	Standard	10 µg	554579
IL-4	Standard	10 µg	550067
IL-5	Standard	5 µg	554581
IL-6	Standard	5 µg	554582
IL-10	Standard	10 µg	550070
GM-CSF	Standard	10 µg	554586
MCP-1	Standard	5 µg	554590
TNF	Standard	10 µg	554589
Rat			
IL-4	Standard	5 µg	555107
IL-10	Standard	5 µg	555113
GM-CSF	Standard	5 µg	555111
MCP-1	Standard	5 µg	555110

Intracellular Cytokine-Positive Control Cells

Description	Cytokines Expressed	Cat. No.
Human		
HiCK 1	Positive for IL-2, IFN-γ, TNF	555061
HiCK 2	Positive for IL-3, IL-4, IL-10, IL-13, GM-CSF	555062
HiCK 3	Positive for IL-1α, IL-1β, IL-6, IL-12, TNF	555063
HiCK 4	Positive for IL-8, GRO-α, IP-10, MCP-1, MCP-3, MIG, MIP-1α	555064
Mouse		
MiCK 1	Positive for IL-2, IFN-γ, TNF	554652
MiCK 2	Positive for IL-3, IL-4, IL-10, GM-CSF, TCA3	554653
MiCK 3	Positive for IL-1α, IL-6, IL-12. MCP-1, TNF	554654
Rat		
RiCK 2	Positive for IL-4, IL-10, GM-CSF, IFN-γ, TNF	555094

Description	Clone	Format	Size	Cat. No.
Isotype Controls				
Mouse IgG_1, κ	MOPC-21	FITC	0.1 mg	554679
		PE	0.1 mg	554680
		PE	100 Tests	559320
		APC	0.1 mg	554681
Mouse IgG_{2a}, κ	G155-178	FITC	0.1 mg	554647
		PE	0.1 mg	554648
		PE	100 Tests	559319
Mouse IgG_{2b}, κ	27-35	FITC	0.1 mg	555057
		PE	0.1 mg	555058
Rat IgG_1	R3-34	FITC	0.1 mg	554684
		PE	0.1 mg	554685
		PE	100 Tests	559318
		APC	0.1 mg	554686
Rat IgG_{2a}, κ	R35-95	FITC	0.1 mg	554688
		PE	0.1 mg	554689
		PE	100 Tests	559317
		APC	0.1 mg	554690
Rat IgG_{2b}	A95-1	FITC	0.1 mg	556923
		PE	0.1 mg	556925
		APC	0.1 mg	556924
Hamster IgG	G235-2356	PE	0.1 mg	554711

Intracellular Cytokine Flow Cytometry Kits and Reagents

Description	Cat. No.
Kits	
BD Cytofix/Cytoperm Kit	554714
BD Cytofix/Cytoperm Kit (with BD GolgiStop)	554715
BD Cytofix/Cytoperm Kit (with BD GolgiPlug)	555028
Human Intracellular Cytokine Staining Starter Kit	559302
Mouse Intracellular Cytokine Staining Starter Kit	559311
FITC BrdU Flow Kit	559619
APC BrdU Flow Kit	552598

Description	Clone	Format	Size	Cat. No.
Related Flow Cytometric Reagents and Buffers				
anti-BrdU	3D4	Purified	0.1 mg	555627
anti-BrdU		FITC Set	100 tests	556028
anti-BrdU		PE Set	100 tests	556029
BD Cytofix/Cytoperm Buffer			125 mls	554722
BD Perm/Wash Buffer (10×)			100 mls	554723
BD GolgiStop (containing monensin)			0.7 ml	554724
BD GolgiPlug (containing brefeldin A)			1.0 ml	555029
BD Cytofix Buffer			100 mls	554655
BD Pharmingen Stain Buffer (FBS)			500 mls	554656
BD Pharmingen Stain Buffer (BSA)			500 mls	554657
BrdU Solution			25.0 mg	550891
7-AAD Staining Solution			2.0 ml	559925
Propidium Iodide Staining Solution			2.0 ml	556463
Related BrdU Products				
BD Pharmingen BrdU *In-Situ* Detection Kit			50 tests	550803
BD Pharmingen BrdU *In-Situ* Detection Kit II			200 tests	551321

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Notes

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