Chapter 1

Immunofluorescent Staining of Cell Surface Molecules for Flow Cytometric Analysis of Immune Function

Introduction

To understand immune responses, it is necessary to identify, isolate, and study a variety of cell types, cell functions, and interactions that constitute those responses. A vast array of different cell surface molecules are involved in mediating immune responses. Methods that determine the types and levels of such membrane molecules (surface markers) that are co-expressed by cells provide important information regarding cell lineage, activation status, adhesion, migration and homing capacity, and ability to respond to stimuli and to interact with other cells. For the purposes of this handbook, this chapter will focus on methods for the detection and measurement of cell surface molecules that mediate cellular functions by virtue of their expression and/or binding of signaling molecules that are critical for cellular intercommunication. Such signaling molecules include cytokines, chemokines, inflammatory mediators, and their receptors, (ie, biological response modifiers [BRMs] of the Immune System). Upon interaction with their specific receptors, BRM ligands can influence the physiology of either the producer cell (autocrine action), adjacent target cells (paracrine action) or distant target cells (endocrine action). In this way, BRMs may influence target cell activation, growth, proliferation, differentiation, migration, and effector function (eg, expression of other BRMs).

Cytokine, Chemokine, and Inflammatory Mediator Receptors

Cytokine receptors are grouped into superfamilies based on the common sequence homologies of their extracellular regions. The main superfamilies recognized today are the Cytokine Receptor (aka, Hematopoietic Receptor), Protein Tyrosine Kinase Receptor, TNF Receptor, Interferon Receptor (aka, Cytokine Receptor Type II), and IL-1/Toll-like Receptor Superfamilies.[1-3] Some receptors consist of a single polypeptide chain that is responsible for both cytokine binding and signal transduction. Other receptors consist of two or more chains, one of which is primarily associated with ligand binding while the other chain(s) is associated with changes in the binding affinity or mediates signal transduction. The high affinity IL-2 receptor serves as an example of a complex receptor with α (CD25), β (CD122), and γ_c (common gamma chain, CD132) subunits that play different roles. Different receptor complexes may also share the same signaling subunit while they consist of different binding subunits. For example, the human β_c subunit (common beta chain, CD131) can combine with distinct receptor subunits specific for IL-3 (IL-3Rα, CD123), IL-5 (IL-5Rα, CD125) or GM-CSF(GM-CSFRα, CD116). Some cytokine receptor subunits are

constitutively expressed by resting cell types and undergo modest upregulation upon cellular activation, while others can be dramatically upregulated (eg, IL-2Rα) by stimulated cells.[4] Cytokine receptors transduce external biological signals into intracellular events by various signal transducing proteins including Protein Tyrosine Kinases.[1–3, 5–7]

Most of the cytokine receptors are transmembrane proteins, although in some cases measurable (even high) levels of circulating, soluble forms (extracellular domains) of the receptors are observed (eg, soluble TNFRs, IL-2Rs, IL-4Rs, and IL-6Rs).[8–10] Soluble cytokine receptors may regulate cytokine actions by specifically binding their cognate cytokine and thus inhibiting its interaction with receptors expressed on target cells.[2] Alternatively, soluble receptors may potentiate the effects of their bound cytokine by extending its half-life in the circulation.[2] The failure to control the levels of circulating cytokines may contribute to pathological situations including sepsis, tissue damage, inflammation, and autoimmunity.

Chemokine receptors belong to the Rhodopsin Superfamily (seven transmembrane receptors) and are G-protein-coupled. Chemokine receptors can be divided into several families based on their ligand specificity, including CXC receptors, CC receptors, CX_3C receptor, and orphan receptors.[1,2]

Inflammatory mediator receptors are very diverse as one would expect given the tremendous variety of ligands. This group of molecules includes receptors that bind products of complement activation cascades such as C3a and C5a fragments.[1] Other inflammatory receptors engage products of the arachidonic pathway (eg, prostaglandins and leukotrienes), specific molecules made by infectious organisms (eg, CD14, Toll-like receptors), or protein mediators (ie, acute phase proteins, granzymes, and defensins).

Biological Response Modifier Receptors and Flow Cytometry

Some receptors (eg, cytokine receptors) are expressed at relatively low levels by unstimulated cells (10 – 1000 molecules/cell), but their surface levels can be considerably upregulated following activation (>10,000 molecules/cell). In certain cases, the level of cell surface receptors remains quite low even after cellular activation, (eg, 100 – 1000 molecules/cell).[11-13] In the past, the measurement of surface BRM receptors expressed by cell populations was made by using a receptor binding assay with radioactively-labeled ligands (radioreceptor assay).[13] Although the radioreceptor assay is useful, it primarily measures high affinity receptors that are often comprised of multiple subunits, not individual receptor subunits. This assay can be successfully used to estimate the numbers of receptors expressed by cells within homogeneous cell populations, such as cell lines. However, it can provide only an average value of receptor levels expressed per cell when the sample is comprised of a mixture of various cell types.

To better understand the physiology of a particular BRM ligand, it is necessary not only to measure its levels in biological fluids (eg, serum, plasma, cell culture supernatants), but also to characterize the frequencies and types of cells that produce the BRM and determine the nature of the target cells that express its

cognate receptors.[14] Multiparameter flow cytometric analysis is a quick, specific, high-throughput method that makes these latter types of studies feasible. Even mixed cell populations, which are routinely prepared from peripheral blood or lymphoid tissues, are amenable to high resolution analysis by using multiparameter flow cytometry.

A large number of fluorescent antibodies specific for cell surface and intracellular markers can be used to characterize cells within populations by multiparameter flow cytometric analysis. In this way, it is possible to gather information regarding each cell's state of activation and differentiation, lineage, migration potential, and functional responsiveness (***Figures 1–4***). For example, it is known that receptors for some cytokines increase upon cellular activation. Evidence of their reduced expression could be indicative of a pathologic condition (eg, HIV infection).[15]

Multicolor flow cytometric analysis also enables analysis of complex cellular interactions in mixed cell populations. For instance, analysis of the expression of cell surface markers or intracellular molecules along with cytokine receptor subunits may provide insights into the potential of individual cells within subsets to produce and/or respond to certain cytokines. This type of analysis allows the researcher to make predictions regarding the types of immune responses that could result from interactions amongst cells within sample populations. These predicted cellular response pathways can then be tested by further experimentation (eg, through the use of differentiation cultures that can generate Th1 versus Th2 types of responses).

A great advantage for cells that can be identified by immunofluorescent staining and flow cytometric analysis in mixed cell populations is that they can also be purified by fluorescent-activated cell sorting or by other means (eg, the BD™ IMag Magnetic Cell Separation System). This can allow isolation of individual cells based on lineage, activation, or cellular differentiation.

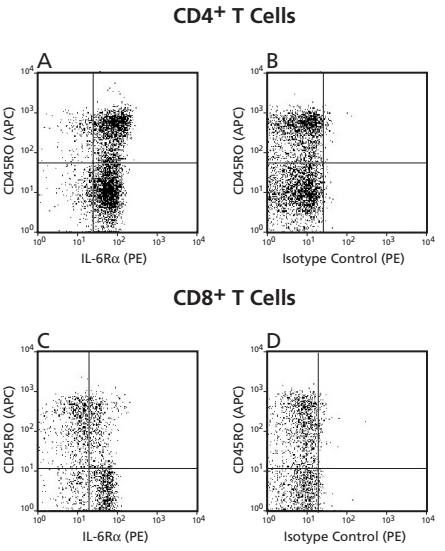

Figure 1. Differential expression of human IL-6Rα (CD126) chain on CD4⁺ and CD8⁺ T cells. Human PBMCs were isolated by density gradient centrifugation (Ficoll-Paque™) and were stained with FITC-anti-human CD4 (Cat. No. 555346, Panels A and B), PerCP-anti-human CD8 (custom made by the Custom Technology Team, BD Biosciences Pharmingen, Panels C and D), APC-anti-human CD45RO (Cat. No. 559865, all Panels) and PE-anti-human IL-6Rα (CD126, Cat. No. 551850, Panels A and C), and PE mouse IgG_1,κ isotype control (Cat. No. 555749, Panels B and D) antibodies. Staining with the anti-human IL-6Rα (CD126) antibody is compared to staining derived with an isotype control antibody (B, D). Two-color dot plots showing the correlated expression patterns of IL-6Rα (CD126) or Ig isotype control and CD45RO were derived from immunofluorescent-gated events with the forward and side light-scatter characteristics of viable CD4⁺ or CD8⁺ lymphocytes.

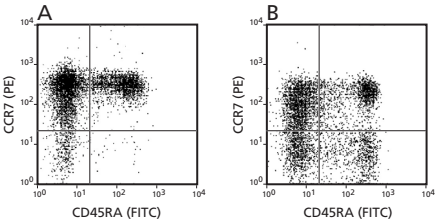

Figure 2. Detection of human CCR7 expression on CD4⁺ and CD8⁺ human lymphocytes by PE-conjugated anti-human CCR7 antibody. Human PBMCs were stained with PE-conjugated anti-human CCR7 (clone 3D12, Cat. No. 552176, Panels A and B) and FITC-conjugated anti-human CD45RA (Cat. No. 555488, Panels A and B). The two-color data shown are derived from the CD4⁺ (based on staining with APC-conjugated anti-human CD4, Cat. No. 555349, Panel A) and CD8⁺ (based on staining with APC-conjugated anti-human CD8, Cat. No. 555369, Panel B) lymphocyte-gated populations.

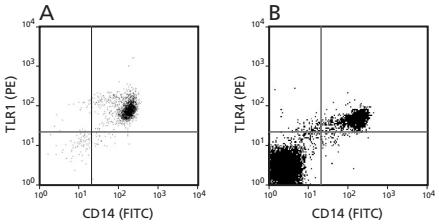

Figure 3. Detection of TLR1 and TLR4 expression on human peripheral blood monocytes. Human peripheral blood mononuclear cells were either treated with BD Pharm Lyse™ (Cat. No. 555899) to lyse red blood cells (Panel A) or were purified by density gradient centrifugation (Ficoll-Paque™) to isolate PBMCs (B). The cells were subsequently stained with either purified anti-human TLR1 (clone GD2, Cat. No. 552033, Panel A), or purified anti-human TLR4 (clone HTA125, Cat. No. 551964, Panel B). The anti-human TLR1 and anti-human TLR4 antibodies were then detected by either biotinylated $F(ab')_2$ goat anti-mouse IgG (Caltag, Cat. No. M35015, Panel A) or biotinylated anti-mouse IgG_{2a} (Cat. No. 553388, Panel B), respectively, followed by PE-streptavidin (Cat. No. 554061, both Panels) and FITC-rat anti-human CD14 (Cat. No. 555397, both Panels). Gates in panel A were set to include cells that were $CD14^+$. The two-color data shown in panel B are derived from ungated mononuclear cell populations.

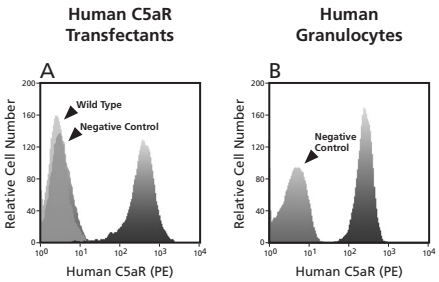

Figure 4. Expression of human C5aR on C5aR transfectants and granulocytes. Human C5aR transfected and wild type mouse L cells were treated with Mouse BD FcBlock™, CD16/CD32 (FcγII/II Receptor, Cat. No. 553141 and 553142) to block Ig Fc- receptors and were stained with PE-conjugated anti-human-C5aR antibody, (clone C85-4124, Cat. No. 552993, Panel A). Human granulocytes were isolated from human peripheral blood by density gradient centrifugation using Polymorphoprep™ (Nycomed). Isolated granulocytes were subsequently stained with PE-conjugated anti-human-C5aR antibody (Panel B) and FITC-conjugated anti-human CD16 (Cat. No. 555406, Panel B). Gates were set to include cells that were $CD16^+$ and had the forward and side light-scatter characteristics of granulocytes (Panel B). Histograms defined as negative control indicate C5aR transfectants (Panel A) or human granulocytes (Panel B) stained with PE streptavidin only.

Other Assays Used to Study BRM Receptor Biology

Multiprobe Ribonuclease Protection Assay (RPA): The multiprobe RPA can be used to measure the mRNA levels for multiple BRM ligands and receptors within cell or tissue lysate samples (described in ***Chapter 11***). The RPA does not provide information concerning the types and levels of transcripts at the level of individual cells. Due to potential post-transcriptional and post-translational modifications, it is desirable to follow up RPA analyses with analyses conducted at the protein level (eg, by immunofluorescent staining and flow cytometric analysis).

Enzyme-Linked Immunosorbent Assay (ELISA): Sandwich ELISAs can be used to quantitate soluble BRM ligands and their receptors that are present in serum, plasma, or in tissue culture supernatants (described in ***Chapters 7 and 8***.)

Biological assays: A variety of bioassays can be used to evaluate whether a test cell population expresses functional BRM receptors by the ability of a test cell population to respond to a given BRM ligand (described in ***Chapter 10***).

Protocol: Multicolor Immunofluorescent Staining for Receptors and Other Cell Surface Antigens.

1. Harvest cells

Viable leukocytes can be obtained from peripheral blood or lymphoid tissues. Activated cell populations can also be prepared from *in vivo*-stimulated tissues or from *in vitro*-activated cultures. Single cell suspensions are prepared and the cell concentrations are adjusted to 2×10^7/ml (for staining in microwell plates; BD Falcon™ Cat. No. 353910) or 10^7/ml (for staining in tubes; BD Falcon 12×75 polystyrene Cat. No. 352008). All incubations and reagents are kept at 4°C with sodium azide to minimize receptor modulation (eg, internalization or shedding). The cells should be protected from light throughout staining and storage.

2. Block Immunoglobulin Fc Receptors

Reagents that block immunoglobulin Fc receptors (FcR) may be useful for reducing nonspecific immunofluorescent staining.

a. In the mouse and rat systems, purified antibodies directed against mouse FcγII/III (Mouse BD FcBlock™, CD16/CD32, Cat. No. 553141 and 553142) and rat FcγIII Receptor (Rat BD FcBlock, CD32, Cat. No. 550270 and 550271) respectively, can be used to block nonspecific staining due to FcR. To block FcR with BD FcBlock reagents, preincubate the cells with 10 µg/ml of BD FcBlock antibody per 2×10^7 cells for 15–20 min at 4°C. The cells are then transferred (10^6 cells/test) to either microwell plates or plastic tubes for immunofluorescent staining. The cells are not washed before the first staining step.

b. FcR on human cells can be pre-blocked by incubating cells (10^6 cells) with human IgG (polyclonal human IgG, Sigma, Cat. No. I–4506). Alternatively, one can use 10% normal human serum in PBS for 20 minutes at 4°C to block Fc receptors.

3. Stain for Receptors and Other Cell Surface Antigens

a. Direct immunofluorescent staining

1. Incubate ~10^6 cells in 100 µl of staining buffer (see Buffers for more information) containing a pre-titrated, optimal concentration (usually ≤ 1 µg) of a fluorescent monoclonal antibody specific for a receptor or with an immunoglobulin (Ig) isotype-matched control for 30 – 45 min at 4°C. In cases of multicolor staining, other fluorescent antibodies directed at various cell surface antigens can be added at the same time with the receptor-specific antibody.

2. After the incubation, add 100 – 200 µl of staining buffer and pellet the cells by centrifugation (250 × *g* for 5 min). Wash the cells 1× with 200 µl of staining buffer, pellet by centrifugation (250 × *g* for 5 min), and remove supernatant.

   For staining in tubes, wash the cells 1× with 2 ml of staining buffer and pellet the cells by centrifugation (250 × *g* for 5 min), and remove supernatant.

3. For staining in microwell plates, add 200 µl of staining buffer to each well, transfer the contents to staining tubes (BD Falcon, 12 × 75mm tubes, Cat. No. 352008) and bring up the volume to 0.5 ml with staining buffer and keep them at 4°C until flow cytometric analysis. For staining in tubes, resuspend cell samples in 0.5 ml of staining buffer and keep them at 4°C until flow cytometric analysis. If desired, cells may be fixed with BD Cytofix Buffer™ (Cat. No. 554655, 100 µl/test) prior to flow cytometric analysis. After fixation, cells are washed as indicated in step 3.a.2 and stored at 4°C until analysis. However, it should be noted that some antigens are sensitive to fixation, resulting in a reduced level of staining (eg, anti-mouse CD21/CD335, clone 7G6).

b. Indirect immunofluorescent staining – 2 Layer Staining

1. Incubate ~10^6 cells in 100 µl with a pre-titrated, optimal concentration (≤ 1 µg) of a purified or biotinylated monoclonal antibody specific for a receptor or with an Ig isotype-matched control antibody for 30 – 45 min at 4°C.

2. After the incubation, add 100–200 µl of staining buffer and pellet the cells by centrifugation (250 × *g* for 5 min). Wash the cells 1× with 200 µl of staining buffer, pellet by centrifugation (250 × *g* for 5 min), and remove supernatant.

   For staining in tubes, wash the cells 1× with 2 ml of staining buffer and pellet the cells by centrifugation (250 × *g*, 5 min), and remove supernatant.

3. Resuspend and incubate cells in 100 µl of staining buffer containing a pre-titrated, optimal concentration (usually ≤ 1 µg per 10^6 cells) of a fluorescent anti-Ig secondary antibody (for troubleshooting see ***Critical Parameters for Detection of Cell Surface Antigens by Flow Cytometry, 7. Background Staining, page 23***) or fluorescent streptavidin (usually ≤ 0.06 µg per 10^6 cells) for 30 min at 4°C.

Note: In cases of multicolor staining, other fluorescent antibodies may be used to detect various cell-surface antigens. When the fluorescent antibodies used for staining additional cell surface antigens originate from the same species as the primary antibody, they have the potential to bind to the fluorescent secondary anti-Ig antibody. To eliminate this possibility, after incubating cells with the fluorescent anti-Ig antibody, wash the cells and then block the unoccupied binding sites of the fluorescent anti-Ig antibody with Ig contained within normal serum obtained from the same species as the primary antibodies (25 µl of neat serum for 20 min). After blocking, add the other fluorescent antibodies and incubate for 20 – 30 min at 4°C.

4. Wash cells as indicated in step 3.b.2.
5. For staining in microwell plates, add 200 µl of staining buffer to each well, transfer the contents to staining tubes and bring up the volume to 0.5 ml with staining buffer and keep them at 4°C until flow cytometric analysis. For staining in tubes, resuspend cell samples in 0.5 ml of staining buffer in tubes and keep them at 4°C until flow cytometric analysis. If desired, cells may be fixed with BD Cytofix Buffer (Cat. No. 554655, 100 µl/test) prior to flow cytometric analysis. After fixation, cells are washed as indicated in step 3.b.2 and stored at 4°C until analysis.

c. Indirect immunofluorescent staining – 3 Layer Staining. For certain BRM receptors that are expressed at very low levels, it may be necessary to use "3 layer" indirect immunofluorescent staining method to "amplify" the fluorescent signal.

1. Incubate ~10^6 cells in or 100 µl of staining buffer containing a pre-titrated, optimal concentration (usually ≤ 1 µg) of a purified monoclonal antibody specific for a receptor or with an Ig isotype-matched control antibody for 30 – 45 min at 4°C.
2. After the incubation, add 100 – 200 µl of staining buffer and pellet the cells by centrifugation (250 × *g* for 5 min). Wash the cells 1× with 200 µl of staining buffer, pellet by centrifugation (250 × *g* for 5 min), and remove supernatant.

   For staining in tubes, wash the cells 1× with 2 ml of staining buffer and pellet the cells by centrifugation (250 × *g* for 5 min), and remove supernatant.
3. Resuspend and incubate cells in 100 µl of staining buffer containing a pre-titrated, optimal concentration (usually ≤ 1 µg) of a biotinylated anti-Ig secondary antibody (for troubleshooting see ***Critical Parameters for Detection of Cell Surface Antigens by Flow Cytometry, 7. Background Staining, Page 23***) for 30 min at 4°C.
4. Wash cells as indicated in step 3.c.2.
5. Resuspend and incubate cells for 30 min at 4°C cells in 100 µl of staining buffer containing a pre-titrated, optimal concentration (usually ≤ 0.25 µg) of a fluorescent streptavidin (eg, phycoerythrin- or allophycocyanin-streptavidin for maximum fluorescent signal intensities and minimal cellular autofluorescence).

Note: In cases of multicolor staining, other fluorescent antibodies may be used to detect various cell surface antigens. When the fluorescent antibodies used for staining additional cell surface antigens originate from the same species as the primary antibody, they have the potential to bind to the biotinylated secondary anti-Ig antibody. To eliminate this possibility, after incubating cells with the biotinylated anti-Ig antibody, wash them and then block the unoccupied binding sites of the biotinylated anti-Ig antibody with Ig contained within normal serum obtained from the same species as the primary antibody (25 µl of neat serum for 20 min). After blocking, add the other fluorescent antibodies and incubate for 20 – 30 min at 4°C.

6. For staining in microwell plates, add 200 µl of staining buffer to each well, transfer the contents to staining tubes and bring up the volume to 0.5 ml with staining buffer and keep them at 4°C until flow cytometric analysis. For staining in tubes, resuspend cell samples in 0.5 ml of staining buffer in tubes and keep them at 4°C until flow cytometric analysis. If desired, cells may be fixed with BD Cytofix Buffer (Cat. No. 554655, 100 µl/test) prior to flow cytometric analysis. After fixation, cells are washed as indicated in step 3.c.2 and stored at 4°C until analysis.

Alternative Protocol: Staining Cell Surface Receptors in Whole Blood.

1. Dilute whole blood 1:10 with (1×) BD Pharm Lyse™ (Cat. No. 555899), mix well, and incubate 10 min at room temperature (RT) in the dark.
2. Spin for 5 min at 500 × *g*.
3. Aspirate supernatant. Wash 2× with 2 ml of staining buffer. Spin for 5 min at 500 × *g*. Aspirate supernatant.
4. Continue with FcR blocking and staining (see S***tain for Receptors and Other Cell Surface Antigens, page 14***).

   Note: The detection of certain cytokine receptors (eg, IL-6R, IL-4R) may be affected by the lysis step if this is performed prior to staining. In those cases, it is recommended to lyse after staining cytokine receptors as indicated below:

   1. Add 100 µl of anti-coagulated whole blood to plastic tubes.
   2. Stain with receptor-specific antibodies (see ***Stain for Receptors and Other Cell Surface Antigens, page 14***).
   3. Wash cells 2× with staining buffer (2 ml/tube), pellet by centrifugation (250 × *g* for 5 min), and remove supernatant.
   4. Resuspend cells with 200 µl BD Pharm Lyse, vortex, incubate at RT for 10 min.
   5. Wash 2× as indicated in step 3 and proceed step a.2 (see ***Stain for Receptors and Other Cell Surface Antigens, pages 14 and 15***).

Staining Controls

1. Positive Staining Controls

Certain cell surface antigens such as cytokine receptors are upregulated upon cell stimulation. The Technical Data Sheets (TDS) for the BD Pharmingen cytokine-receptor-specific antibodies may describe *in vitro* culture systems that induce detectable frequencies of cytokine-receptor-expressing cells at specific timepoints. Cells stimulated by these methods can be used as positive controls for experimental systems. Alternatively, cell lines that are widely available may also be recommended in the TDS. For those receptor subunits that are constitutively expressed, unstimulated cells can be used as controls.

2. Negative Staining Controls

The following controls can be used to discriminate specific from nonspecific staining:

a. **Staining of a negative cell population:** Staining of a cell line or a specific cell subset within a mixed cell population that is known not to express a specific receptor chain can serve as a negative staining control.

b. **Immunoglobulin isotype control:** Stain with an immunoglobulin (Ig) isotype control of irrelevant specificity. Stain as described in the aforementioned procedure for receptors and other cell surface antigens. Ig isotype controls should be used at the same concentration as the receptor-specific antibody.

c. **Blocking antibody control: Preincubate cells with unconjugated antibody.** This type of negative control can only be used for fluorescent or biotinylated receptor-specific antibodies.

   1. Resuspend cells in 50 µl of staining buffer (50 µl for staining in tubes) containing unconjugated receptor-specific antibody (same clone as conjugated antibody) diluted to be in excess when compared to the conjugated antibody (usually 5 µg/10^6 cells), and incubate 30 min at 4°C.
   2. After incubation, add fluorescent or biotinylated receptor-specific antibody at an optimal concentration in 50 µl of staining buffer for a final volume of 100 µl, and incubate 30–45 min at 4°C.
   3. Wash cells (see ***Stain for Receptors and Other Cell Surface Antigens, page 15, 3.b.2 – 3.b.5***)

   Note: The purified antibody should significantly (>90%) block staining by the fluorescent or biotinylated antibody that subsequently is added to cells.

3. Other Controls

The following controls can be used to optimize instrument settings:

a. Autofluorescence controls

Autofluorescence results from fluorescent emissions occurring when intracellular materials are excited at the same wavelength as the fluorescent probes used for staining. *In vitro*-cultured cells, tumors, or cells high in granule content may have relatively higher autofluorescence when compared with other cells. To determine the baseline fluorescence of each cell population studied, controls that include only unstained (ie, not stained for the marker of interest) cells can be used.

b. Compensation controls

Electronic compensation may be necessary to correct the spectral overlap of fluorescent emissions when multiple fluorescent probes excited by a single wavelength are used. Cell samples stained with individual fluorescent probes (ie, two fluorescent antibodies such as FITC- and PE-conjugated antibodies) can be compared with cells labeled with both fluorescent probes to determine the level of fluorescence signal overlap and to establish proper compensation. For more detailed information see ***reference 19***.

Buffers

Staining Buffer

- Dulbecco's PBS (DPBS)
- 2% heat inactivated FCS
- 0.09% (w/v) sodium azide
- Adjust buffer pH to 7.4 – 7.6, filter (0.2 µm pore membrane), and store at 4°C.

BD Biosciences offers two buffers: BD PharmingenStain™ (FBS) (Cat. No. 554656) and BD PharmingenStain™ (BSA) (Cat. No. 554657) that are rigorously tested for their ability to optimize immunofluorescent staining and maintain cell viability.

Critical Parameters for Detection of Cell Surface Antigens by Flow Cytometry.

1. Stimulation and Harvesting of Cells

Certain cell surface antigens such as cytokine receptors (eg, mouse IL-12Rβ2) are expressed in very low numbers in non-stimulated cells but can be upregulated up to ten-fold higher levels following cell activation. Therefore, it is necessary to determine the cell activation conditions that enhance their surface expression (***Figure 5***). In other cases, the level of surface expression for an antigen may decline after cell activation due to shedding (eg, certain cytokine receptors).[17] Use of inhibitors in the tissue culture medium that block receptor shedding has been used successfully to reverse this effect (eg, see ***TNFRI and TNFRII***), (***Figure 6***).

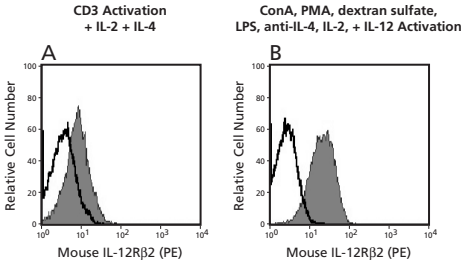

Figure 5. Mouse IL-12Rβ2 expression on in vitro-activated cells. C57BL/6 mouse splenocytes were treated to lyse erythrocytes and were cultured for 5 days with either plate-bound anti-mouse CD3 antibody (Cat. No. 553057) plus recombinant mouse IL-2 (Cat. No. 554578) and IL-4 (Cat. No. 550067) (Panel A) or with ConA (2 µg/ml), PMA (5 ng/ml), dextran sulfate (10 µg/ml), LPS (5 µg/ml), anti-IL-4 antibody (5 µg/ml, Cat. No. 554432), recombinant mouse IL-2 (10 ng/ml, Cat. No. 550069) and IL-12 (20 ng/ml Cat. No. 554592) (Panel B). Five days later, cells were harvested, washed and blocked with mouse BD FcBlock (10 µg/ml, Cat. No. 553141, both Panels). Cells were subsequently stained with purified anti-mouse IL-12Rβ2 (clone HAM10B9, Cat. No. 552819, both Panels) followed by PE-labeled anti-hamster IgG (cocktail) (Cat. No. 554056, both Panels) and BD Via-Probe™ (Cat. No. 555815, both Panels). Staining with the anti-mouse IL-12Rβ2 antibody (filled histograms) is compared to staining obtained using a Purified Hamster IgG_1, κ Isotype control (Cat. No. 553951, both Panels, open histograms). Histograms were derived from gated events of viable (7-AAD negative) lymphocytes.

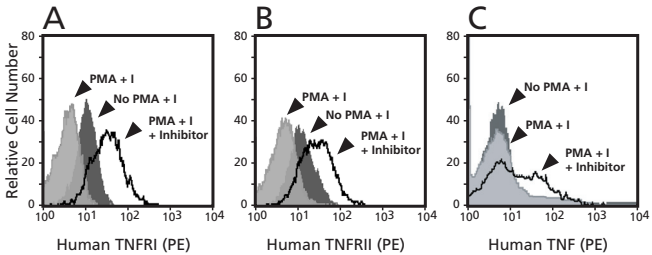

Figure 6. TACE (Tumor Necrosis Factor-Alpha Converting Enzyme) inhibitors block activation-induced shedding of TNFRs and membrane TNF. Human PBMCs isolated by density gradient centrifugation (Ficoll-Paque™) were stimulated with plate-bound anti-human CD3 antibody (10 µg/ml, Cat. No. 555336) and soluble anti-CD28 antibody (2 µg/ml, Cat. No. 555725) in the presence of human IL-2 (10 ng/ml, Cat. No. 554603) and IL-4 (40 ng/ml, Cat. No. 554605) for 2 days. The cells were subsequently washed and expanded in IL-2 and IL-4 for three days. Following expansion, the cells were washed and stimulated for 2 hr with PMA (5 ng/ml) and ionomycin (500 ng/ml) with or without 25 µM of metalloprotease inhibitors (TAPI) or were used without further stimulation. Following incubation, the cells were harvested and their surface expression of human TNFRI and TNFRII were detected by immunofluorescent staining and flow cytometric analysis using biotinylated anti-human TNFRI (clone MABTNFR1-B1, Cat. No. 550900, Panel A) and purified anti-human TNFRII (clone hTNFR-M1, Cat. No. 551311, Panel B), respectively. The anti-human TNFRI and anti-human TNFRII antibodies were subsequently detected with PE-streptavidin (Cat. No. 554061, Panel A) and biotinylated $F(ab')_2$ goat anti-rat IgG (Jackson ImmunoResearch, Cat. No. 112-066-062, Panel B) followed by PE-streptavidin, respectively. Expression of membrane TNF was detected using the PE-labeled anti-human TNF antibody (clone MAb11, Cat. No. 559321, Panel C). Histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes.

2. Quality of Antibody

   The choice of high affinity, receptor-specific antibodies for immunofluorescent staining is very critical. Certain antibody isotypes may be problematic because they tend to nonspecifically bind to FcRs. For example, antibodies with the mouse IgG_1 isotype tend to nonspecifically bind less than other mouse and rat Ig isotypes to surface FcRs expressed by human PBMCs.

3. Choice of Protocol—Direct versus Indirect Staining

   In cases where direct immunofluorescent staining is employed, high sensitivity can be achieved using phycoerythrin- or allophycocyanin-conjugated antibodies. Phycoerythrin (PE) and allophycocyanin (APC) have high extinction coefficients (the efficiency of conversion of excitation energy to fluorescence energy) and therefore give better quantum yields than most other commercially available fluorochromes.[11,13] Therefore, in multicolor flow cytometric analysis for cytokine receptors and other cell surface antigens, it is recommended that PE- and APC-labeled antibodies be used for staining antigens that are expressed at relatively low levels. Fluorescein isothiocyanate (FITC)- and PerCP-labeled reagents should be used for staining antigens that are coexpressed at relatively higher levels.

   The limit of sensitivity for flow cytometry is typically around 200 – 500 molecules/cell (depending on the nature of the cells, reagents, staining protocol and flow cytometer that is used). Sensitivity is defined as the significant separation between the signal from positive cells when compared with signals given by negative cell controls. For those receptors that are expressed at such low levels, signal amplification can be achieved by increasing the "layers" of immunofluorescent staining.[11,13] For example, use of biotinylated, polyclonal secondary antibodies followed by PE- or APC-streptavidin ("3 layer staining") has proven to be the preferred method for increased sensitivity (***Figure 7***).

   Each primary antibody can theoretically be bound by at least two secondary antibodies, each one of which carries several biotin molecules (which in turn can bind PE- or APC-streptavidin).

   Note: PerCP-labeled reagents are not recommended for immunofluorescent staining of cells that are used for sorting because they tend to photobleach after excitation by the high energy laser excitation used by cell sorters.

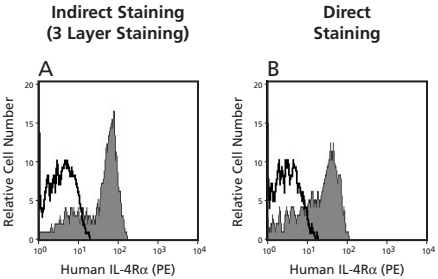

Figure 7. Analysis of IL-4Rα chain expression on human B cells. Human PBMCs were isolated by density gradient centrifugation (Ficoll-Paque™) and were treated with human IgG (5 µg/10^6 cells) to block Ig Fc receptors. The cells were subsequently stained with either purified anti-human IL-4R (clone hIL4R-M57, Cat. No. 551850, Panel A) antibody followed by biotinylated anti-mouse IgG_1 (Cat. No. 553441, Panel A) and PE-streptavidin (Cat. No. 554061, Panel A) or PE anti-human IL-4R (clone hIL4R-M57, Cat. No. 552178, Panel B). Samples were blocked with mouse serum (25 µl/10^6 cells) and stained with FITC anti-human CD19 antibody (clone HIB19, Cat.No. 555412, both Panels). Staining with the anti-human IL-4R antibody (filled histograms) is compared to staining obtained using a Mouse IgG_1, κ isotype control antibody (Cat. No. 555746, both Panels, open histograms). Histograms were derived from gated events with the forward and side light-scatter characteristics of viable $CD19^+$ lymphocytes.

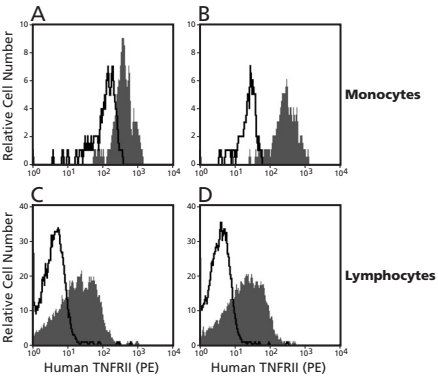

Figure 8. Effect of FcR blocking on the analysis of TNFRII expressed by human PBMCs. Human PBMCs were isolated by density gradient centrifugation (Ficoll-Paque™) and were treated with with human IgG (5 µg/10^6 cells, Panels B and D) to block FcR. The cells were subsequently stained with purified anti-human TNFRII (clone hTNFR-M1, Cat. No. 551311, all Panels) followed by biotinylated $F(ab')_2$ goat anti-rat IgG (Jackson ImmunoResearch, Cat. No. 112-066-062, all Panels) and PE-streptavidin (Cat. No. 554061, all Panels). Staining with the anti-human TNFRII antibody (filled histograms) is compared to staining obtained using a Rat IgG_{2b}, κ isotype control antibody (Cat. No. 553986, all Panels, open histograms). Histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes and monocytes.

4. Antigen Modulation and Receptor Internalization

   Certain surface antigens, such as cytokine receptors, may be susceptible to internalization or shedding (eg, mouse TNFRI). Therefore, shortly after cell harvesting for immunofluorescent staining, it is necessary to minimize cell handling at room temperature and carry out all incubations at 4°C. To further prevent antigen modulation and internalization, it is recommended that the metabolic inhibitor sodium azide be used in the staining buffer.

5. FcR Blocking

   To eliminate or reduce non-specific binding of antibodies caused by FcR, cells should be pretreated with FcR-blocking reagents. For example, in the mouse and rat systems, specific monoclonal antibodies are available that are directed against FcγII/III and FcγII receptors respectively. They have been proven to successfully reduce non-specific immunofluorescent staining caused by FcRs. In the human system, an excess of purified Ig from human or other species (or autologous serum that contains Ig) can be used (***Figure 8***).[18] Alternatively, fragmented $F(ab')_2$ antibodies may be available that can be used for immunofluorescent staining.

6. Immunoglobulin Isotype Controls

   Certain antibody isotypes have a greater tendency than others to bind non-specifically to FcRs. To extract meaningful conclusions from experiments that involve immunofluorescent staining, it is recommended that Ig isotype-matched controls be run in the same experiment at the same dose as the antigen-specific antibodies. Ideally, if the test antibodies are conjugated, the isotype controls must be conjugated in the same way.

7. Background Staining

   In cases of indirect immunofluorescent staining where a two- or three-layer staining protocol is employed, the secondary anti-Ig reagent might cross-react with cell-surface immunoglobulin of the species being studied. To eliminate such background staining, the use of monoclonal isotype-specific anti-Ig secondary reagents (rather than polyclonal antibody preparations) or $F(ab')_2$ secondary antibodies are recommended (***Figure 9***). Frequently, it is necessary to screen a number of secondary anti-Ig reagents for sensitivity versus background staining before choosing the most suitable secondary reagent.

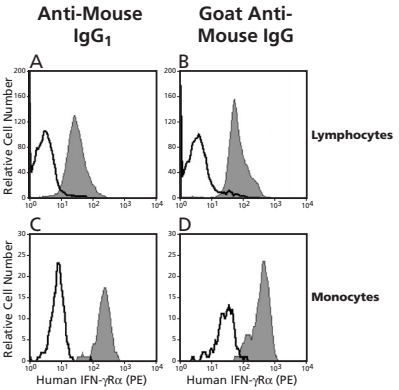

Figure 9. Analysis of IFN-γRα chain expression on human PBMCs. Human PBMCs were isolated by density gradient centrifugation (Ficoll-Paque™) and were stained with purified anti-human IFN-γRα (clone GIR-208, Cat. No. 558932, all Panels) followed with either biotinylated anti-mouse IgG_1 (Cat. No. 553441, Panels A and C) or biotinylated goat anti-mouse IgG (Cat. No. 553999, Panels B and D) and PE-streptavidin (Cat. No. 554061, all Panels). Staining with the anti-human IFN-γRα antibody (filled histograms) is compared to staining obtained using a Mouse IgG_1, κ isotype control antibody (Cat. No. 555746, all Panels, open histograms). Histograms were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes (Panels A and B) and monocytes (Panels C and D).

8. Cell Viability

   Cell viability is particularly an issue when dealing with cultured cells. Dead cells tend to aggregate and nonspecifically adsorb fluorescent antibodies. Large numbers of dead cells in cell suspensions can be removed by centrifugation on density separation media (eg, Ficoll-Paque™, Pharmacia). Smaller numbers of dead cells can be excluded from the flow cytometric analysis by using either propidium iodide (Propidium Iodide Solution, Cat. No. 556463) or 7–AAD (BD Via-Probe™, Cat. No. 555815).[18]

9. Data Analysis

   Single parameter data files can be displayed as histograms (frequency distributions) with fluorescence intensity on the *x*-axis and relative cell number on the *y*-axis. Using appropriate software, single-parameter data can also be displayed as overlapping histograms. The percentages of positive cells can be calculated by the placement of a marker (eg, whose placement is determined due to unstained, Ig-isotype-stained, or stained negative cell controls) or by channel-by-channel subtraction methods when histograms are overlaid. Alternatively, bivariate (two-parameter) plots of light scatter signals and fluorescence intensities can be generated for single-color (as well as multicolor) immunofluorescent staining and flow cytometric experiments. Bivariate plots can be displayed in either a dot plot or a contour plot format with parameter intensities on the *x*- and *y*-axes. In this case, positive and negative controls should be compared to identify specific areas of staining so that quadrant markers or other gates can be applied to enumerate the frequencies of cells that coexpress the two parameters in a particular manner. For more details on data analysis please refer to unit 5.2 of ***Current Protocols in Immunology***.[19]

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Cytokine, Chemokine, and Inflammatory Mediator Receptor Antibodies for Flow Cytometry

Description	Clone	Isotype	Format	Cat. No.
Human Cytokine Receptors				
Common β chain (CDw131)	3D7	Mouse IgG_1	Purified	554534
			Biotin	554535
Common γ chain (CD132)	TUGh4	Rat IgG_{2b}	Purified	555896
			Biotin	555897
			PE	555898
Common γ chain (CD132)	AG184	Mouse IgG_1	PE	555900
EGFR	EGFR.1	Mouse IgG_{2b}	Purified	555996
			PE	555997
Endoglin (CD105)	266	Mouse IgG_1	Purified	555690
G-CSFR (CD114)	LMM741	Mouse IgG_1	Purified	554536
			Biotin	554537
			PE	554538
GM-CSFRα (CDw116)	hGMCSFR-M1	Mouse IgG_1	Purified	551284
			Biotin	551412
			PE	551373
GM-CSFRα (CDw116)	M5D12	Mouse IgM	Purified	554530
			Biotin	554531
			FITC	554532
gp130 (CD130)	AM64	Mouse IgG_1	Purified	555756
			PE	555757
IFN-γRα (CD119)	GIR-208	Mouse IgG_1	Purified	558932
			PE	558934
IFN-γRα (CD119)	GIR-94	Mouse IgG_{2b}	Purified	558935
			PE	558937
IGF-I Rα (CD221)	1H7	Mouse IgG_1	Purified	555998
			PE	555999
IGF-I Rα (CD221)	3B7	Mouse IgG_1	Purified	556000
IL-2Rα (CD25)	M-A251	Mouse IgG_1	Purified	555430
			FITC	555431
			PE	555432
			PE-Cy5	555433
			APC	555434
IL-2Rβ (CD122)	Mik-β2	Mouse IgG_{2a}	Purified	554520
			Biotin	554521
			PE	554522
IL-2Rβ (CD122)	Mik-β3	Mouse IgG_1	Purified	554523
			Biotin	554524
			PE	554525
IL-3Rα (CD123)	9F5	Mouse IgG_1	Purified	555642
			Biotin	555643
			PE	555644
IL-3Rα (CD123)	7G3	Mouse IgG_{2a}	Purified	554527
			Biotin	554528
			PE	554529
IL-4Rα (CD124)	hIL4R-M57	Mouse IgG_1	Purified	551894
			Biotin	552120
			PE	552178
IL-5Rα (CD125)	A14	Mouse IgG_1	Purified	555901
			PE	555902

Cytokine, Chemokine, and Inflammatory Mediator Receptor Antibodies for Flow Cytometry

Description	Clone	Isotype	Format	Cat. No.
Human Cytokine Receptors *(continued)*				
IL-6Rα (CD126)	M5	Mouse IgG_1	Purified	551462
			Biotin	551851
			PE	551850
IL-10R (CD210)	3F9	Rat IgG_{2a}	Purified	556012
			PE	556013
IL-12Rβ1 (CD212)	2.4E6	Mouse IgG_1	Purified	556064
			PE	556065
IL-12Rβ2 (CD212)	2B6/12β2	Rat IgG_{2a}	Purified	550722
			PE	550723
IL-18Rα	H44	Mouse IgG_1	Purified	552951
LIFR	12D3	Mouse IgG_1	Purified	559790
			PE	559791
LIFR	7G7	Mouse IgG_1	Purified	559778
			PE	559779
Lymphotoxin β Receptor (TNFR Related Protein)	hTNFR-RP-M12	Mouse IgG_1	Purified	551359
			Biotin	551861
			PE	551503
	BCG6.AF5	Mouse IgG_1	Purified	552875
NGFR	C40-1457	Mouse IgG_1	Purified	557194
			Biotin	557195
			PE	557196
PDGFRa (CD140α)	αR1	Mouse IgG_{2a}	Purified	556001
			PE	556002
PDGFRβ (CD140β)	28D4	Mouse IgG_{2a}	Purified	558820
			PE	558821
SCFR (CD117, c-kit)	YB5.B8	Mouse IgG_1	Purified	555713
			PE	555714
			PE-Cy5	559879
			APC	550412
TNF Receptor type I (CD120a)	MABTNFR1-B1	Mouse IgG_{2a}	Purified	550514
			Biotin	550900
TNF Receptor type II (CD120β)	hTNFR-M1	Rat IgG_{2b}	Purified	551311
			Biotin	552417
			PE	552418
			APC	552419
4-1BB (CDw137)	4B4-1	Mouse IgG_1	Purified	555955
			PE	555956
			PE-Cy5	551137
			APC	550890
Human Chemokine Receptors				
CCR5 (CD195)	2D7/CCR5	Mouse IgG_1	Purified	555991
			FITC	555992
			PE	555993
			APC	556903
			PE-Cy5	556889
CCR5 (CD195)	3A9	Mouse IgG_{2a}	Purified	556041
			PE	556042
			APC	550856

Cytokine, Chemokine, and Inflammatory Mediator Receptor Antibodies for Flow Cytometry

Description	Clone	Isotype	Format	Cat. No.
Human Chemokine Receptors *(continued)*				
CCR6	119A	Mouse IgG_1	Purified	559560
			Biotin	559561
			PE	559562
CCR7	2H4	Mouse IgM	Purified	550937
CCR7	3D12	Rat IgG_{2a}	Purified	552175
			Biotin	552174
			PE	552176
CXCR1 (CD128a, IL-8RA)	5A12	Mouse IgG_{2b}	Purified	555937
			Biotin	555938
			FITC	555939
			PE	555940
CXCR2 (CDw128b,IL-8RB)	6C6	Mouse IgG_1	Purified	555932
			FITC	551126
			PE	555933
			APC	551127
			PE-Cy5	551125
CXCR3 (CD183)	1C6/CXCR3.1	Mouse IgG_1	Purified	557183
			PE	557185
			APC	550967
			PE-Cy5	551128
CXCR4 (CD184, Fusin)	12G5	Mouse IgG_{2a}	Purified	555972
			Biotin	555973
			PE	555974
			APC	555976
			PE-Cy5	555975
CXCR4 (CD184, Fusin)	1D9	Rat IgG_{2a}	Purified	551413
			Biotin	551970
			PE	551510
CXCR5	RF8B2	Rat IgG_{2b}	Purified	552032
			Biotin	552118
Human Inflammatory Mediators and their Receptors				
CD14	M5E2	Mouse IgG_{2a}	Purified	555396
			FITC	555397
			PE	555398
			APC	555399
CD21	1048	Mouse IgG_1	Purified	552727
C1qRp	R139	Mouse IgG_{2b}	Purified	551087
			FITC	551531
			PE	551509
C1qRp	R3	Mouse IgM	Purified	551454
			Biotin	552117
C3a receptor	8H1	Mouse IgG_1	Purified	557173
C5a receptor	C85-4124	Rabbit IgG	Purified	559159
			PE	inquire
C5a receptor	D53-1473	Mouse IgG_1	Purified	550493
			PE	550494

Cytokine, Chemokine, and Inflammatory Mediator Receptor Antibodies for Flow Cytometry

Description	Clone	Isotype	Format	Cat. No.
Human Inflammatory Mediators and their Receptors *(continued)*				
fMLP receptor	5F1	Mouse IgG_1	Purified	556015
			PE	556016
Leucotriene B4 receptor (hBLTR)	203/14F11	Mouse IgG_1	Purified	552400
			Biotin	552835
			PE	552836
Thioredoxin	polyclonal	Goat IgG	Purified	550483
			PE	556016
Thioredoxin (full length, TRX 1-1)	2G11	Mouse IgG_1	Purified	559969
			FITC	559968
Thioredoxin (full length, TRX 1-8)	7D11(TRX4)	Mouse IgG_1	Purified	559966
			Biotin	550887
Human Cell Surface Cytokines				
Lymphotoxin-α (LTα2β1)	BF7.AE2	Mouse IgG_1	Purified	inquire
Lymphotoxin-α (LTα1β1)	AG9.BD6	Mouse IgG_1	Purified	552873
Lymphotoxin-β	B9.C9	MouseIgG_1	Purified	551359
TNF	MAb11	Mouse IgG_1	Purified	554510
			Purified	559071
			FITC	554512
			PE	554513
			PE	559321
			APC	554514
Toll-like Receptors				
Toll-like Receptor 1 (TLR1)	GD2	Mouse IgG_1	Purified	552033
Toll-like Receptor 4 (TLR4)	HTA125	Mouse IgG_1	Purified	551964
			Biotin	551975

See complete listing for Human Complement Receptors in ***Chapter 12.***

Mouse Cytokine Receptors				
CD131 (bIL-3R, β common)	JORO50	Rat IgG_1	Purified	559918
			Biotin	559919
			PE	559920
Common γ chain (CD132)	4G3	Rat IgG_{2a}	Purified	554455
			Biotin	554456
			PE	554457
Common γ chain (CD132)	TUGm2	Rat IgG_{2b}	Biotin	554470
			PE	554471
IFN-γRα (CD119)	GR20	Rat IgG_{2a}	Purified	558770
			Biotin	558771
IFN-γRα (CD119)	2E2	Arm. Hamster IgG	Purified	559911
			Biotin	550482
IFN-γRβ	MOB-47	Arm. Hamster IgG	Purified	559917
IL-1R I (CD121a)	35F5	Rat IgG_1	Purified	553693
			Biotin	550969
			PE	557489
IL-1R I (CD121a)	12A6	Rat IgG_{2a}	Purified	557490
IL-1R II (CD121b)	4E2	Rat IgG_{2a}	Purified	554448
			Biotin	554449
			PE	554450

Cytokine, Chemokine, and Inflammatory Mediator Receptor Antibodies for Flow Cytometry

Description	Clone	Isotype	Format	Cat. No.
Mouse Cytokine Receptors *(continued)*				
IL-2Rα (CD25)	7D4	Rat IgM	Purified	553068
			Biotin	553069
			Biotin	553070
			FITC	553071
			FITC	553072
IL-2Rα (CD25)	3C7	Rat IgG_{2b}	Purified	557364
			PE	553075
IL-2Rα (CD25)	PC61	Rat IgG_1	Purified	557425
			PE	553866
			APC	557192
			PerCP-Cy5.5	551071
			PE-Cy7	552880
IL-2Rβ (CD122) (IL-2/-15Rβ)	TM-β1	Rat IgG_{2b}	Purified	557461
			Biotin	559884
			FITC	553361
			PE	553362
IL-2Rβ (CD122)	5H4	Rat IgG_{2a}	Purified	554451
			FITC	554452
IL-3Rα (CD123)	5B11	Rat IgG_{2a}	Purified	555069
			Biotin	555070
			PE	555071
IL-4Rα	mIL4R-M1	Hamster IgG	Purified	551853
			Biotin	552508
			PE	552509
IL-6Rα (CD126)	D7715A7	Rat IgG_{2b}	Purified	554460
			Biotin	554461
			PE	554462
IL-7Rα (CD127)	B12-1	Rat IgG_{2a}	Purified	558776
			Biotin	555288
IL-7Rα (CD127)	SB/14	Rat IgG_{2a}	Purified	550425
			PE	550479
IL-7Rα (CD127)	SB/199	Rat IgG_{2b}	PE	552543
IL-10R (CD210)	1B1.3a	Rat IgG_1	Purified	559912
			Biotin	559913
			PE	559914
IL-12Rβ1 (CD212)	114	Mouse IgG_{2a}	Purified	551455
			Biotin	551973
			PE	551974
IL-12Rβ2	HAM10B9	Hamster IgG	Purified	552819
Lymphotoxin β Receptor	AF.H6	Hamster IgG	Purified	552940
Lymphotoxin β Receptor	AC.H6	Hamster IgG	Purified	552939
PDGFRα (CD140a)	APA5	Rat IgG_{2b}	Purified	558774
SCFR (CD117, c-kit)	2B8	Rat IgG_{2b}	Purified	553352
			Biotin	553353
			FITC	553354
			PE	553355
			APC	553356
SCFR (CD117, c-kit)	ACK45	Rat IgG_{2b}	Purified	553868
			PE	553869
TNF receptor type I (CD120a)	55R-286	Arm. Hamster IgG	Purified	559915
TNF receptor type II (CD120b)	TR75-89	Arm. Hamster IgG	Purified	559916
			Biotin	550476
			PE	550086

Cytokine, Chemokine, and Inflammatory Mediator Receptor Antibodies for Flow Cytometry

Description	Clone	Isotype	Format	Cat. No.
Mouse Chemokine Receptors				
CCR3	Polyclonal	Rabbit IgG	Purified	556882
CCR5 (CD195)	C34-3448	Rat IgG_{2c}	Purified	559921
			Biotin	559922
			PE	559923
CXCR4 (CD184, Fusin)	2B11/CXCR4	Rat IgG_{2b}	Purified	551852
			Biotin	551968
			FITC	551967
			PE	551966
CXCR5	2G8	Rat IgG_{2a}	Purified	551961
			Biotin	551960
			PE	551959
Mouse Cell Surface Cytokines				
Lymphotoxin-α	AF.B3	Hamster IgG	Purified	552937
Lymphotoxin-β	BB.F6.F6.BF2	Hamster IgG	Purified	552938
TNF	MP6-XT22	Rat IgG_1	Purified	554416
			Purified	559064
			FITC	554418
			PE	554419
			APC	554420
TNF	TN3-a9.12	Hamster IgG	PE	559503
Mouse Inflammatory Mediator Receptors				
CD14 (LPS Receptor)	rmC5-3	Rat IgG_1	Purified	553738
			FITC	553739
			PE	553740
CR2/CR1 (CD21/CD35)	7G6	Rat IgG_{2b}	Purified	533817
			FITC	553818
			PE	552957
C5a receptor	C1150-32	Polyclonal Rabbit IgG	Purified	552837

See complete listing for Mouse Complement Receptors in ***Chapter 12****.*

Rat Cytokine Receptors				
IL-2Rα (CD25)	OX-39	Mouse IgG_1	Purified	559980
			Biotin	559981
			FITC	554865
			PE	554866
Rat Cell Surface Cytokines				
TNF	TN3-19.12	Hamster IgG	PE	559503

See complete listing for Rat Complement Receptors in ***Chapter 12****.*

Note: Please see the 2003 BD Biosciences Product Catalog for more information concerning reagents that recognize:

- *TNF Superfamily Ligands and Receptors, including Fas, FasLigand, CD40, CD40 Ligand, etc.*
- *BRM molecules expressed by cells from other species including Non-Human Primates, Pigs, Rabbits, and Dogs.*

Reagents for Immunofluorescent Staining of Cell Surface Molecules

For an updated list of antibodies and other reagents for immunofluorescent staining of cell surface molecules, please refer to the BD Biosciences online product catalog website at **www.bdbiosciences.com** or contact BD Biosciences Technical Services at **877.232.8995** for a copy of the latest BD Biosciences Catalog.

Notes