

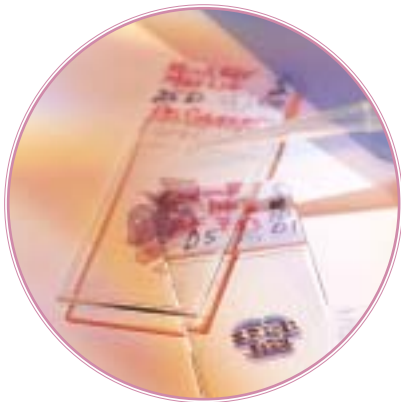
Antibodies

R&D Systems offers a wide range of antibodies to cytokines, adhesion molecules, proteases and developmental proteins. Monoclonal and polyclonal antibodies are available, labeled or unlabeled. The majority of polyclonal antibodies are supplied as antigen affinity-purified IgG preparations. Each antibody is quality controlled to ensure lot-to-lot consistency and outstanding performance in a variety of applications.



“We find the affinity-purified polyclonal antibodies from R&D Systems to be very reliable laboratory reagents. The antibodies work at relatively high dilutions making them fairly inexpensive reagents to use on a test-by-test basis.”

Dr. David F. Lappin
University of Glasgow
Dental Hospital and School
Glasgow, UK



“We have used R&D Systems’ polyclonal antibodies extensively for several years in immunofluorescence studies in paraffin sections from renal biopsies. When used in combination with an appropriate IgG control on serial sections and scanning laser confocal microscopy, we have been able to achieve semi-quantitative analysis of numerous chemokines, cytokines and receptors, including RANTES and TGF- β .”

Dr. Helen Robertson
University of Newcastle
Department of Pathology
Newcastle, UK

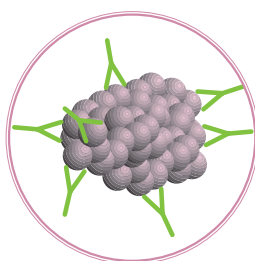


Antibodies

Antibodies Available

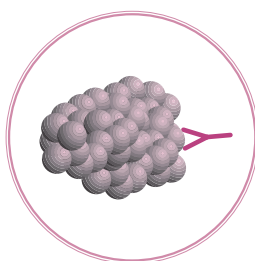
Polyclonal

Polyclonal antibodies offer multiple epitope recognition. Anti-sera and whole immunoglobulin (Ig) preparations contain a large amount of non-specific Ig, which could lead to non-specific antibody binding. Antigen column affinity chromatography eliminates non-specific IgG making these specific IgG preparations, in general, 10-fold more potent.



Monoclonal

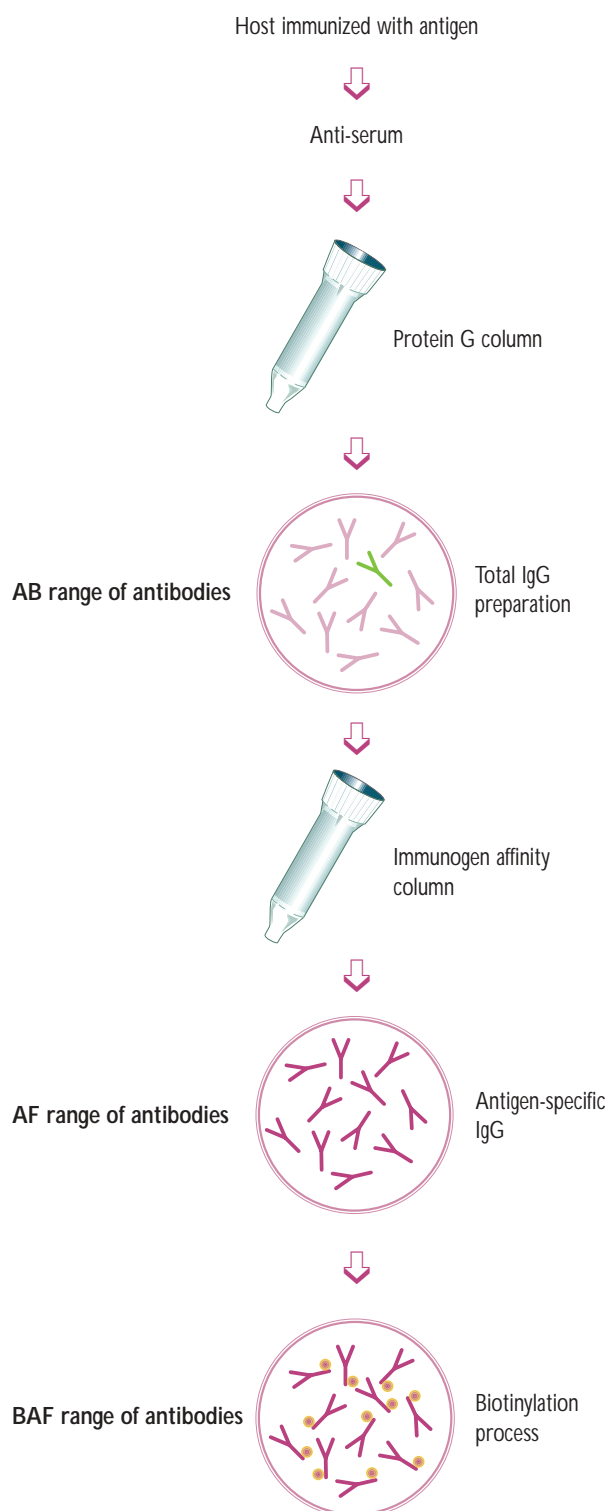
Monoclonal antibodies offer monospecificity, recognizing a single epitope. All new R&D Systems' monoclonal antibodies are derived from hybridoma culture supernatants rather than ascites fluid, and are purified using either protein A or protein G columns.



Labeled Antibodies

A range of both monoclonal and polyclonal antibodies are available conjugated to either biotin, fluorescein or phycoerythrin. Biotinylated antibodies eliminate the need for secondary antibodies and offer the flexibility to use any detection system for which streptavidin or avidin conjugates are available.

Fluorochrome labeled antibodies are designed for flow cytometry detection and quantitation of intracellular or cell surface associated molecules.



Features and Benefits

The range of over 3000 antibody products is available for a variety of animal species: human, mouse, rat, cotton rat, porcine, canine, feline, rhesus macaque, drosophila and viral.

Each antibody is manufactured under controlled conditions, undergoing rigorous quality control testing to ensure lot-to-lot consistency and outstanding performance.

Each new batch of antibody is tested to ensure low endotoxin levels which is reported on individual datasheets. The formulation of most antibodies does not contain azide or other preservative.

For maximum stability most antibodies are supplied lyophilized. This facilitates easy shipping and storage.

All antibody products have a specific datasheet which contains essential information and suggested conditions and concentrations for various applications.

All antibodies are tested for cross-reactivity with other molecules by direct ELISA to ensure specificity.



RnDSystems.com Resources



Product datasheets Each antibody datasheet is available on the web site in pdf format.

Suggested protocols Protocols for common applications are available, including immunohistochemistry, Western blot and neutralization.

Images The site has an extensive selection of in-house immunohistochemistry staining images for reference.

Troubleshooting guide To help with any technical difficulties, troubleshooting guides are available for Immunohistochemistry and Western blot.

LivePerson For questions needing a more immediate answer, LivePerson is available whereby you can 'chat', live on the web, with one of our technical correspondents.

Weekly updates To keep you up to date with the latest antibody releases, you can opt to receive a weekly notification by email. To benefit from this service, please visit our web site and click on [Subscribe](#).

Antibodies

Applications – Immunohistochemistry

Our Immunohistochemistry (IHC) Laboratory screens antibodies for suitability for use in IHC applications, selecting only the antibodies that give the best signal to background ratio. A representative tissue is selected and a standard protocol used to assess both monoclonal and polyclonal antibody suitability.

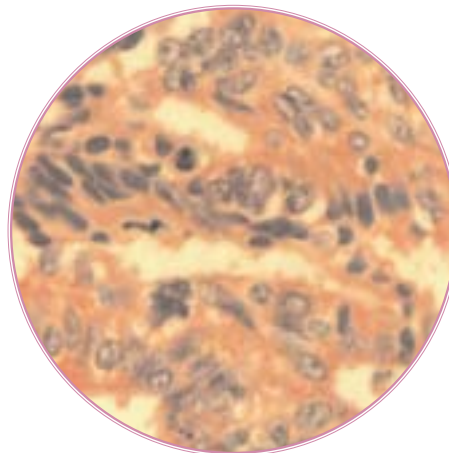
Antigen affinity-purified polyclonal antibodies maximise the opportunity of antigen binding in tissue, due to multiple epitope recognition, but without the drawback of conventional polyclonal antibodies – non-specific binding.

Detailed IHC protocols have been developed and optimized by our in-house laboratory, using frozen and paraffin-embedded tissues and either chromogenic or fluorescent detection methods. The protocols are available on our web site, and can be modified to suit your tissue sample. Paraffin-embedded tissues may require antigen retrieval, depending on the tissue type, fixation protocol and storage.

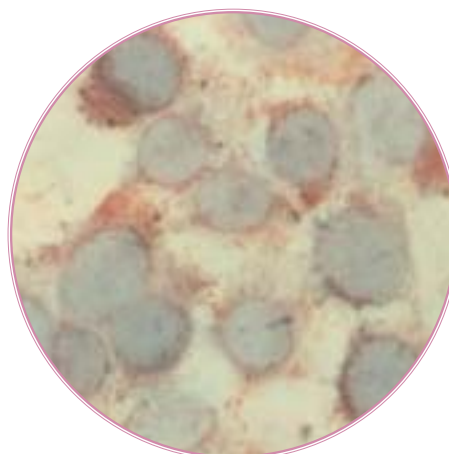
Images of IHC staining are available on the web site. Each image is accompanied by details of the protocol used, antibody concentration and antigen retrieval (if used).

Additional Reagents

R&D Systems' Cell and Tissue Staining Kits have been developed for localization of antigens in a broad range of specimen types, including cytospin preparations. These kits are in a ready-to-use format and include pre-diluted secondary biotinylated antibodies and high sensitivity streptavidin-HRP that saves time and reduces the risk of errors. Kits are available for the detection of goat, rabbit or mouse primary antibodies. Monoclonal and polyclonal control antibodies are also available, as well as antigen retrieval reagents, mounting medium and DAB enhancer.



Monoclonal anti-human IL-8 (MAB208) was used to stain cytoplasmic IL-8 in paraffin-embedded human colorectal tumor, using alkaline phosphatase-FAST RED detection reagents. *Courtesy of R. Brew, Dept of Immunology, University of Liverpool, UK.*



Monoclonal anti-human G-CSF (MAB214) was used to stain bladder carcinoma cell line 5637. A 25 fold dilution was used on acetone fixed cells and detected using alkaline phosphatase reagents. *Courtesy of M. Field, Dept. of Medicine, Royal Infirmary, Glasgow, UK.*

Applications – Western Blot

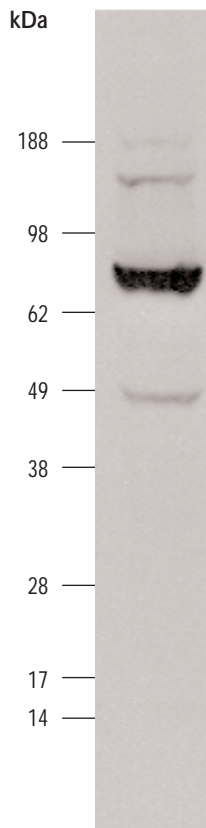
The majority of both monoclonal and polyclonal antibodies are validated for use in Western blot. In-house quality control uses Western blot applications to show that antibodies are sensitive and specific for the protein to which they are raised. Proteins are run under reducing and non-reducing conditions and the sensitivity of detection is quoted on the product datasheet.

Antigen affinity-purified polyclonal antibodies (Cat. # AFXXX) offer maximum opportunity to detect your sample through multiple epitope recognition, without the drawback of conventional polyclonal antibodies, non-specific binding. Many of these antibodies are also available biotinylated, eliminating the need for a secondary antibody, and offering the flexibility to choose your preferred detection system.

A suggested protocol for Western blot is available on our web site, together with an extensive troubleshooting guide and on-line technical support.

Additional Reagents

Polyclonal and monoclonal IgG preparations are available for use as negative controls in Western blots. Molecular weight markers are also available biotinylated or pre-stained blue.



Lysate from 5×10^5 U937 cells was separated on 4–12% Tris-bis precast gel from Invitrogen, then Western blot was run using R&D Systems' anti-human/mouse cIAP-1 polyclonal antibody (Cat. # AF818) at a dilution of 1:1000 (at a final concentration of 1.0 $\mu\text{g}/\text{mL}$).

Courtesy of Dr. Jun Kuai, Ph.D., Wyeth Research, Cambridge, MA 02140, USA

Applications – ELISA Development

R&D Systems offers a wide range of matched antibody pairs intended for ELISA development. Each pair has been tested to ensure:

- compatibility in forming a sandwich with the analyte
- stability once opened and reconstituted
- no crossreactivity with related molecules
- parallel detection of natural protein with the recombinant standard

Detection antibodies are offered biotinylated for flexibility to choose any colorimetric, fluorescent or chemiluminescent detection system for which streptavidin conjugates are available.

A detailed protocol using the HRP:TMB detection system is available on the datasheet and in our *ELISA Development Guide*. This comprehensive booklet contains advice on assay optimization and a troubleshooting guide.

Additional Reagents

Streptavidin-HRP and TMB substrate reagents.

Antibodies

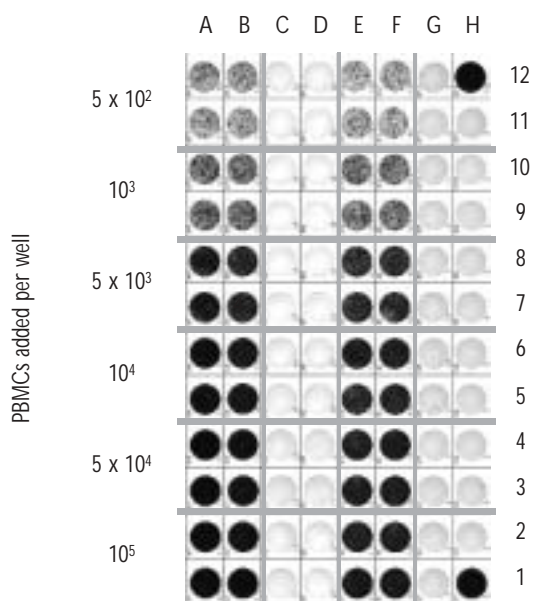
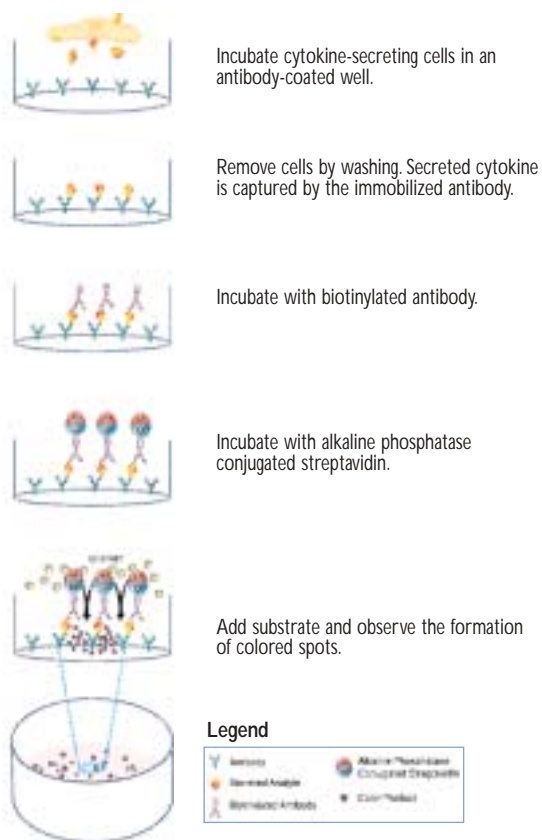
Applications – ELISpot

ELISpot is a sensitive technique used to detect individual cytokine producing cells which allows detection levels that may not be achieved in conventional ELISA or by other techniques.

Either a monoclonal or polyclonal antibody is coated onto a PVDF membrane-backed microplate, into which the cells under investigation are added. The secreted cytokine of interested is captured by the immobilized antibody in the vicinity of the cell. After a suitable incubation time, the cells are removed and the secreted cytokine is detected by a detection antibody and detection reagents. Each spot represents a single positive cell.

R&D Systems offers a wide range of monoclonal, polyclonal and labeled antibodies for hundreds of analytes. This allows selection of a pair of antibodies for use in ELISpot.

Selection of a biotinylated detection antibody eliminates the need for secondary antibodies and allows the flexibility to choose a detection system for which a streptavidin conjugate is available. Alkaline phosphatase used with BCIP/NBT is most commonly used.



A, B – stimulated cells, complete development
 C, D – stimulated cells, detection antibodies were omitted
 E, F – non-stimulated cells, complete development
 G, H – culture medium (no cells)
 H1 and H12 – positive control

Quality Control

R&D Systems' antibodies do not contain azide which may adversely affect cells in the assay. Also, each lot is quality control tested to ensure low endotoxin levels, minimizing stimulation of cells in the assay.

Each antibody is tested for specificity to ensure it detects only the antigen to which it was raised.

Additional Reagents

Recombinant proteins for use as positive controls. Blue Color Module containing alkaline phosphatase conjugated to streptavidin and BCIP/NBT substrate.

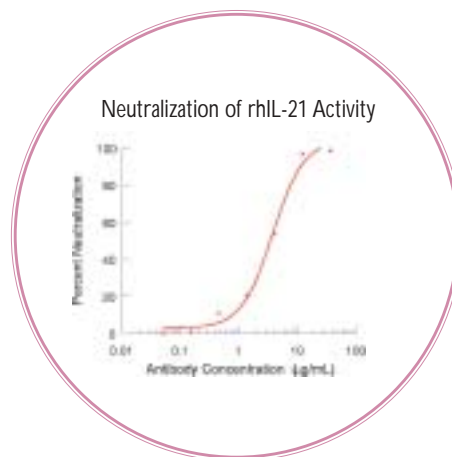
Applications – Neutralization

Neutralizing a particular protein with antibodies can give valuable information about a protein's biological activity, whether it is used *in vivo* or *in vitro*.

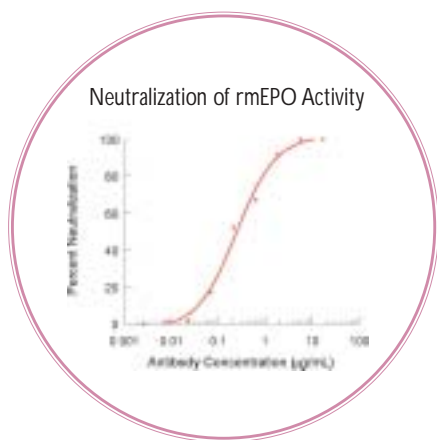
Neutralization is also essential in non-specific bioassays when measuring the biological activity of samples containing several bioactive proteins.

Most of R&D Systems' antibodies are tested for their ability to neutralize the biological activity of their target protein. To test neutralization, a concentration of protein, just high enough to elicit a maximum biological response, is incubated with increasing concentrations of antibody. The concentration of antibody which causes 50% neutralization of the biological response is termed the ND₅₀. Low ND₅₀ values indicate potent neutralization. Each new lot of antibody is assayed to ensure the ND₅₀ value is consistent between lots.

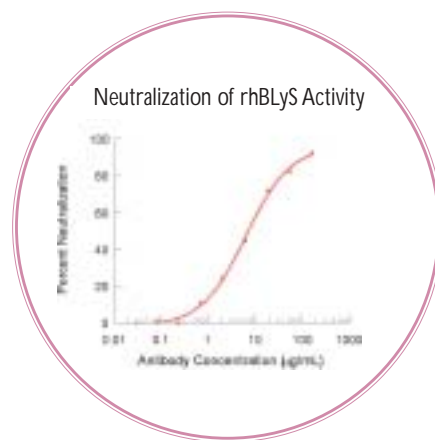
Details of the bioassay used and the neutralization effect are stated on all product datasheets for neutralizing antibodies. Low endotoxin, no preservatives, and high antibody specificity ensure that the observed neutralization of bioactivity is specifically due to antibody binding of target protein.



The ND₅₀ for AF794 (anti human IL-21) was determined to be approximately 3–10 µg/mL in the presence of 125 ng/mL of rhIL-21, using the N-1186 cell line which proliferates in response to IL-21. Resazurin was added for the final 24 hours of the 4 day incubation to measure cell proliferation.



The ND₅₀ of monoclonal anti mouse EPO (MAB959) was determined to be approximately 0.2–0.8 µg/mL in the presence of 40 ng/mL of rmEPO using the factor-dependent cell line TF-1 which proliferates in response to rmEPO. ³H-thymidine was added for the final 4 hours of a 72-hour incubation to determine proliferation.



The ND₅₀ of the monoclonal anti human BLYS (MAB124) was determined to be approximately 2–10 µg/mL when using rhBLYS at 2.5 ng/mL and 4 x 10⁶ primary B cells which proliferate in response to rhBLYS. ³H-thymidine was added for the final 16 hours of a 72-hour incubation to measure cell proliferation.

Antibodies

Applications – Flow Cytometry

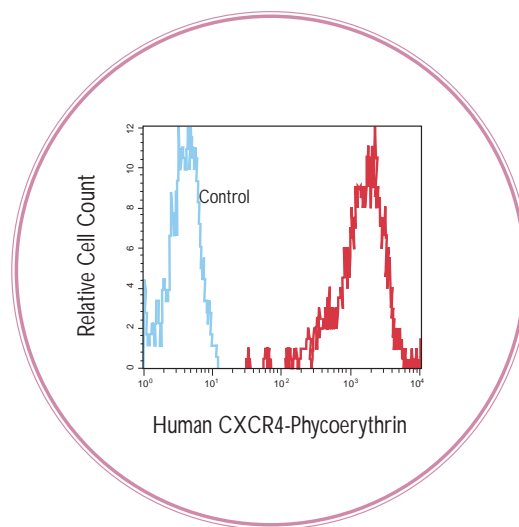
Flow cytometry enables the measurement of certain physical and chemical characteristics of cells as they travel in suspension one by one past a laser. Rapid analysis of large numbers of cells, analysis of several parameters on the same cell, and the collection of quantitative data on a single cell are some of the advantages that flow cytometric analysis offers over other technologies. R&D Systems offers a wide range of monoclonal antibodies conjugated to different fluorochromes that allow for simultaneous multiparameter analysis. These directly conjugated monoclonal antibodies are designed for monitoring the presence of cytokines or cytokine receptors on either cell surfaces or intracellularly. Cell surface staining is useful in the immunophenotyping of cell populations, while intracellular cytokine staining has been utilized to identify cells secreting selected cytokines, e.g. Th1 vs Th2 cells. Intracellular staining antibodies are selected from a panel of monoclonals for their specific recognition of pro- or cytoplasmic forms of molecules.

All directly conjugated antibodies can also be used to perform quantitative flow cytometry analysis to estimate the number of molecules expressed on each cell.

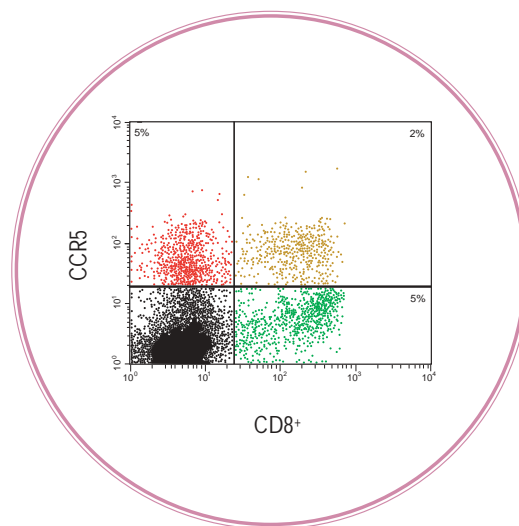
Monoclonal antibodies are preferred for their defined binding characteristics. Certain surface antigens, such as CD34 or CXCR4 exist as different isoforms. Due to epitope monospecificity monoclonals can be used to identify different isoforms of the same antigen, therefore we offer several different clones.

CXCR4 on many cell lines and primary cells exhibits conformational heterogeneity. The antibody clone most commonly used to study CXCR4 expression (I2G5) (MAB170) recognises only a subpopulation of CXCR4 molecules on T cells and freshly isolated B cells. As a result, levels of CXCR4 on these cells have often been underestimated.

A variety of fluorochrome labeled isotype matched controls, conjugated to the same fluorochrome/protein ratio, are also available from R&D Systems as an aid in assessing the background staining of cell populations.



Reactivity of Jurkat cells stained with phycoerythrin-conjugated anti-human CXCR4 monoclonal antibody (Catalog # FAB173P, Clone # 44717), or an appropriate phycoerythrin-conjugated mouse IgG_{2B} isotype control (IC004P).



Reactivity of CD8⁺ peripheral blood lymphocytes stained with phycoerythrin-conjugated anti-human CCR5 monoclonal antibody (Catalog # FAB180P).