

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD45 antibodies
4. References

1. Description

Components	1 mL monoclonal CD45 antibodies, mouse conjugated to various dyes.												
	<table border="0"> <tr> <td>FITC</td> <td>130-091-609</td> </tr> <tr> <td>PE</td> <td>130-091-610</td> </tr> <tr> <td>APC</td> <td>130-091-811</td> </tr> <tr> <td>VioBlue®</td> <td>130-092-910</td> </tr> <tr> <td>VioGreen™</td> <td>130-097-294</td> </tr> <tr> <td>PerCP</td> <td>130-094-962</td> </tr> </table>	FITC	130-091-609	PE	130-091-610	APC	130-091-811	VioBlue®	130-092-910	VioGreen™	130-097-294	PerCP	130-094-962
FITC	130-091-609												
PE	130-091-610												
APC	130-091-811												
VioBlue®	130-092-910												
VioGreen™	130-097-294												
PerCP	130-094-962												
Clone	30F11.1 (isotype: rat IgG2b).												
Capacity	100 tests or up to 10 ⁹ total cells.												
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.												
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.												

1.1 Background information

The mouse CD45 (Ly-5, leukocyte common antigen) antigen is expressed at high levels on all cells of hematopoietic origin, except erythrocytes, and can be used to discriminate leukocytes from nonhematopoietic cells.¹⁻³ The CD45 antibody clone 30F11 reacts with all CD45 isoforms.

1.2 Applications

- Identification and enumeration of CD45⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse leukocytes can be isolated by using, for example, CD45 (L3T4) MicroBeads, mouse (# 130-049-201).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD45 conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD45 MicroBead-labeled cells use the same dilution.

The antibody is suited for staining of formaldehyde-fixed cells.

1.4 Reagent requirements

- **Buffer:** Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
 - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

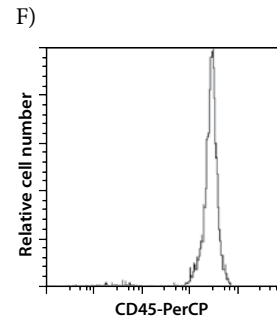
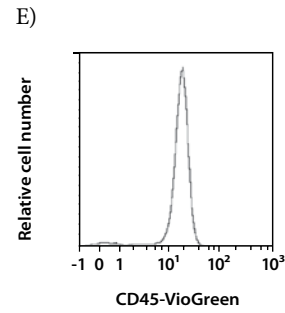
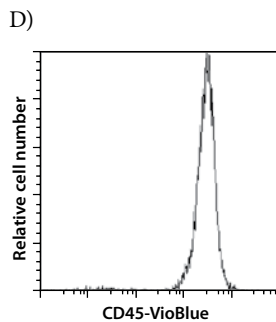
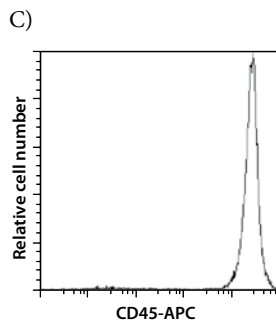
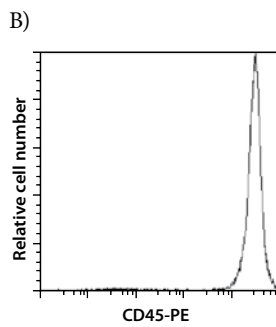
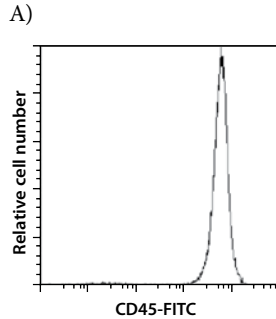
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD45 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD45 antibodies

Mouse splenocytes were stained with CD45 antibodies conjugated to FITC (A), PE (B), APC (C), VioBlue (D), VioGreen (E), or PerCP (F) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Ewijk, W. *et al.* (1981) Fluorescence analysis and anatomic distribution of mouse T lymphocyte subsets defined by monoclonal antibodies to antigens Thy-1, Lyt-1, Lyt-2, and T-200. *J. Immunol.* 127: 2594–2604.
2. Ledbetter, J. A. and Herzenberg, L. A. (1979) Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev.* 47: 63–90.
3. Scheid, M. and Triglia, D. (1979) Further specificity of the Ly-5 system. *Immunogenetics* 9: 423–433.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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