

### Contents

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### 1. Description

<b>Components</b>	1 mL monoclonal CD45R (B220) antibodies, mouse conjugated to various dyes.																
	<table border="0"> <tr> <td>VioBlue®</td> <td>130-094-287</td> </tr> <tr> <td>VioGreen™</td> <td>130-096-909</td> </tr> <tr> <td>FITC</td> <td>130-091-829</td> </tr> <tr> <td>PE</td> <td>130-091-828</td> </tr> <tr> <td>APC</td> <td>130-091-843</td> </tr> <tr> <td>PerCP</td> <td>130-094-966</td> </tr> <tr> <td>PE-Vio770™</td> <td>130-097-031</td> </tr> <tr> <td>APC-Vio770</td> <td>130-096-598</td> </tr> </table>	VioBlue®	130-094-287	VioGreen™	130-096-909	FITC	130-091-829	PE	130-091-828	APC	130-091-843	PerCP	130-094-966	PE-Vio770™	130-097-031	APC-Vio770	130-096-598
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APC	130-091-843																
PerCP	130-094-966																
PE-Vio770™	130-097-031																
APC-Vio770	130-096-598																
<b>Clone</b>	RA3-6B2 (isotype: rat IgG2a).																
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.																
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.																
<b>Storage</b>	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 CD45R (B220) antigen is expressed on B lymphocytes throughout their development from early pro-B stages on and is down-regulated upon terminal differentiation to plasma cells.<sup>1,2</sup> Apart from B cells, CD45R is expressed on a small subset of dendritic cells (plasmacytoid dendritic cells).<sup>2,3</sup> The CD45R monoclonal antibody clone RA3-6B2 specifically recognizes the exon A–restricted isoform of mouse CD45. CD45R is present on approximately 45–55% of spleen, lymph node, and bone marrow cells. It is absent in thymus, but reported to be present on apoptotic thymocytes.<sup>4</sup>

### 1.2 Applications

- Identification and enumeration of CD45R (B220)<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy, for example:
  - positive selection or depletion of mouse B cells by using CD45R (B220) MicroBeads (# 130-049-501), or CD19 MicroBeads (# 130-052-201);
  - isolation of untouched resting mouse B cells by using the B Cell Isolation Kit (# 130-090-862);
  - positive selection or depletion of mouse B-1 cells by using CD5 (Ly-1) MicroBeads (# 130-049-301);
  - isolation of CD11c<sup>+</sup>CD45R<sup>+</sup>mPDCA-1<sup>+</sup> plasmacytoid dendritic cells using the Plasmacytoid Dendritic Cell Isolation Kit II (# 130-092-786).

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD45R (B220) conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD45R (B220) 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 Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) For information about antibodies for additional staining refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (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.

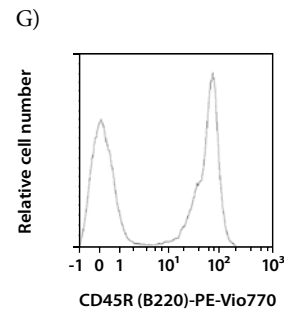
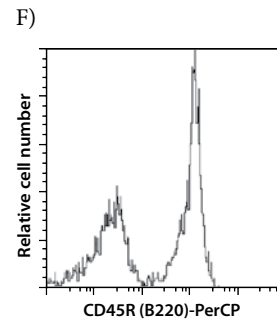
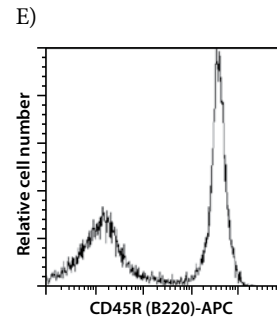
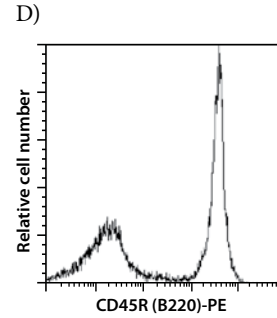
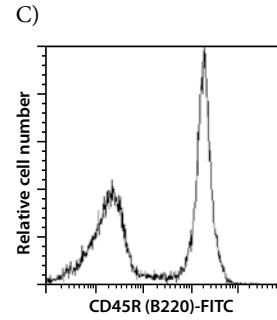
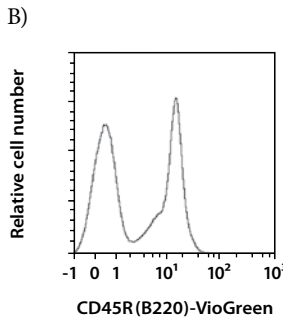
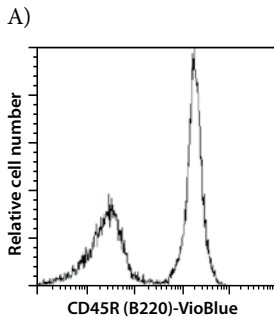
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  nucleated cells. When working with fewer than  $10^7$  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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

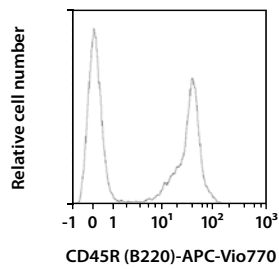
1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the CD45R (B220) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).  
▲ **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  $300 \times g$  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 CD45R (B220) antibodies

Mouse spleen cells were stained with CD45R (B220) antibodies conjugated to VioBlue (A), VioGreen (B), FITC (C), PE (D), APC (E), PerCP (F), PE-Vio770 (G), or APC-Vio770 (H) and analyzed by flow cytometry using the MACSQuant<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



H)



#### 4. References

1. Coffman, R. L. (1982) Surface antigen expression and immunoglobulin gene rearrangement during mouse pre-B cell development. *Immunological Rev.* 69: 5–23.
2. Asselin-Paturel, C. *et al.* (2001) Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat. Immunol.* 2: 1144–1150.
3. Nakano, H. *et al.* (2001) CD11c(+)B220(+)/Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J. Exp. Med.* 194: 1171–1178.
4. Oka, S. *et al.* (2000) Presence of B220 within thymocytes and its expression on the cell surface during apoptosis. *Immunology* 100: 417–423.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

#### Warranty

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