

### 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 CD90.2 antibodies
4. References

### 1. Description

<b>Components</b>	1 mL monoclonal CD90.2 antibodies, mouse conjugated to various dyes.	
	FITC	130-091-602
	PE	130-091-601
	APC	130-091-790
	VioBlue®	130-094-361
	VioGreen™	130-097-295
	PerCP	130-094-959
<b>Clone</b>	30-H12 (isotype: rat IgG2b).	
<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 CD90 (Thy1.2) antigen is expressed at high levels on thymocytes and peripheral T cells in lymphoid organs and blood,<sup>1,2</sup> and at lower levels on early hematopoietic stem cells in bone marrow<sup>3</sup>, interepithelial cells (dendritic epidermal T cells) in skin<sup>4</sup>, and on neurons<sup>5</sup>. The CD90.2 antibody clone 30-H12 reacts with the Thy-1.2 alloantigen, which is a pan T cell marker in the most common inbred mouse strain. It does not cross-react with Thy-1.1. CD90 is present on approximately 30% of splenocytes, 80% of lymph node cells, and almost all thymocytes in healthy mice.

#### 1.2 Applications

- Identification and enumeration of CD90.2<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse T cells can be isolated by using, for example, CD90.2 MicroBeads, mouse (# 130-049-101) or the Pan T Cell Isolation Kit II, mouse (# 130-095-130) for the isolation of untouched T cells.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD90.2 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 CD90.2 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) 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.

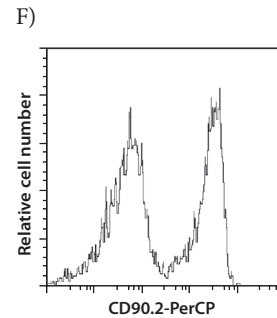
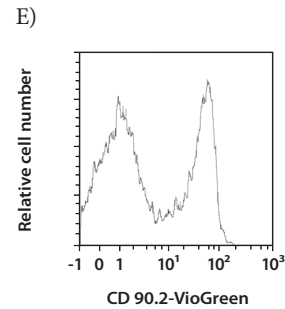
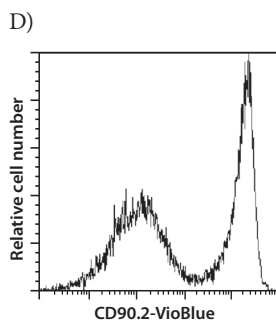
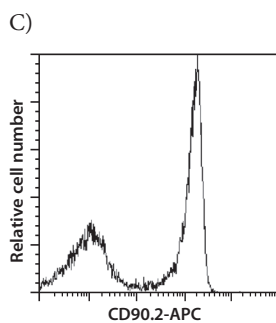
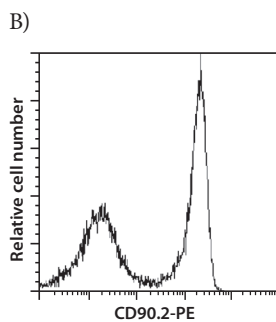
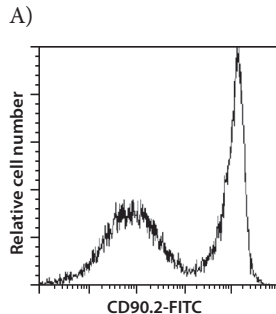
### 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> 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 2×10<sup>7</sup> 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 10<sup>7</sup> nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD90.2 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 CD90.2 antibodies

Mouse spleen cells were stained with CD90.2 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. Ledbetter, J. A. and Herzenberg, L. A. (1979) Xenogenic Monoclonal Antibodies to Mouse Lymphoid Differentiation Antigens. *Immunol. Rev.* 47: 63–90.
2. Ledbetter, J. A. *et al.* (1980) T Cell Subsets Defined by Expression of Lyt-1,2,3 and Thy-1 antigens. *J. Exp. Med.* 152: 280–295.
3. Basch, R. S. and Berman, J. W. (1982) Thy-1 determinants are present on many murine hematopoietic cells other than T cells. *Eur. J. Immunol.* 12: 359–364.
4. Bergstresser, P. R. *et al.* (1985) Origin and function of Thy-1<sup>+</sup> dendritic epidermal cells in mice. *J. Invest. Dermatol.* 85: 85–90.
5. Radrizzani, M. *et al.* (1995) Developmental regulation of Thy 1.2 rate of synthesis in the mouse cerebellum. *J. Neurosci. Res.* 42: 220–227.

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

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, MACSQuant, and VioBlue are registered trademarks and VioGreen is a trademark of Miltenyi Biotec GmbH.

Copyright © 2012 Miltenyi Biotec GmbH. All rights reserved.