

CD90.1 antibodies mouse and rat

CD90.1-FITC	130-094-527
CD90.1-PE	130-094-528
CD90.1-APC	130-094-525
CD90.1-VioBlue™	130-094-588
CD90.1-Biotin	130-094-526
CD90.1 pure	130-094-524

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 CD90.1 antibodies
4. References

1. Description

Components	1 mL CD90.1 antibodies, mouse and rat: monoclonal CD90.1 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), VioBlue™, or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	His51 (isotype: mouse IgG2a).
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 monoclonal antibody His51 reacts with rat CD90 (Thy-1) and mouse CD90.1 (Thy1.1), a GPI-anchored conserved membrane glycoprotein. In the rat, the CD90 antigen is expressed on thymocytes¹, recent thymic emigrants¹, hematopoietic stem cells², on neurons such as retinal ganglion cells^{3,4}, and on other cell types. In the mouse strains AKR/J, PL, and FVB/N, CD90.1 is a pan T cell marker⁵ and can be found on thymocytes, hematopoietic stem cells in the bone marrow, intraepithelial cells (dendritic epidermal T cells) in skin⁶, and on neurons, such as retinal ganglion cells⁷. The antibody does not cross-react with CD90.2 (Thy1.2).

1.2 Applications

- Identification and enumeration of CD90.1⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse or rat T cells can be isolated by using, for example, CD90.1 MicroBeads (# 130-094-523).

1.3 Recommended antibody dilution

For antibody labeling of mouse and rat cells.

CD90.1 conjugate	FITC	PE	APC	VioBlue	Biotin
Flow cytometry^a					
- In general	1:11	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11	1:11	1:11
- CD90.1 MicroBead-labeled cells	1:11	1:11	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.

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 or rat serum albumin, mouse or rat serum, or fetal bovine serum. 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 CD90.1 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

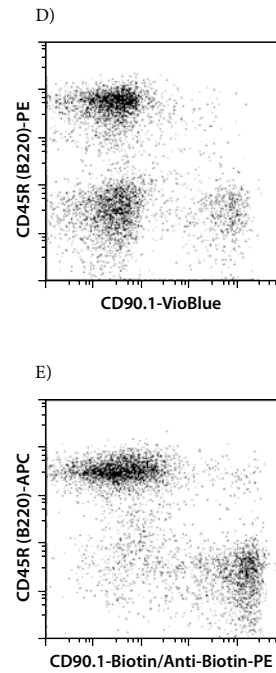
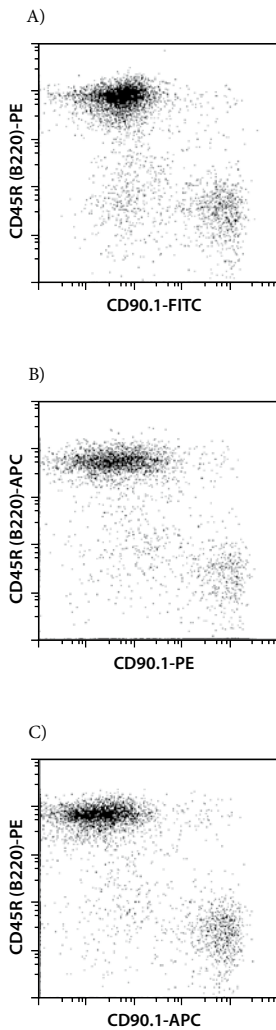
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD90.1-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD90.1 antibodies

Mouse spleen cells were stained with CD90.1 antibodies conjugated to FITC (A), PE (B), APC (C), or VioBlue™ (D) as well as with CD45R (B220)-PE (# 130-091-828) or CD45R (B220)-APC (# 130-091-843) and analyzed using the MACSQuant™ Analyzer. Cells labeled with CD90.1-Biotin (E) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD45R (B220)-APC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

For information about staining of rat spleen cells see www.miltenyibiotec.com.



4. References

1. Hosseinzadeh, H. *et al.* (1993) Recent thymic emigrants in the rat express a unique antigenic phenotype and undergo post-thymic maturation in peripheral lymphoid tissues. *J. Immunol.* 150: 1670–1679.
2. Hermans, M. H. *et al.* (1991) In situ visualization of hemopoietic cell subsets and stromal elements in rat and mouse bone marrow by immunostaining of frozen sections. *J. Histochem. Cytochem.* 39: 1627–1634.
3. Liefer, D. *et al.* (1984) Monoclonal antibody to Thy-1 enhances regeneration of processes by rat retinal ganglion cells in culture. *Science* 224: 303–306.
4. Campbell, D. G. *et al.* (1981) Rat brain Thy-1 glycoprotein. The amino acid sequence, disulphide bonds and an unusual hydrophobic region. *Biochem. J.* 195: 15–30.
5. Houston, J. J. *et al.* (1980) Specific in vivo localization of monoclonal antibodies directed against the Thy 1.1 antigen. *J. Immunol.* 125: 837–743.
6. Bergstresser, P. R. *et al.* (1985) Origin and function of Thy-1+ dendritic epidermal cells in mice. *J. Invest. Dermatol.* 85: 85–90.
7. Su, Y. *et al.* (2008) Axonal regeneration after optic nerve crush in Nogo-A/B/C knockout mice. *Mol. Vis.* 14: 268–273.

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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