



# CD16/32 antibodies mouse

CD16/32-PE	130-092-572
CD16/32-APC	130-092-573
CD16/32-Biotin	130-092-570
CD16/32 pure	130-092-574

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## 1. Description

<b>Clone</b>	93 (isotype: rat IgG2a).
<b>Product format</b>	1 mL CD16/32 antibodies, mouse: monoclonal CD16/32 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.  Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
<b>Product size</b>	100 tests or up to 10 <sup>9</sup> total cells.
<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 and product applications

The CD16/32 antibody recognizes a shared epitope common to the CD16 and CD32 antigens. CD16 and CD32 are also known as FcγIII and FcγII, respectively, and bind to epitopes located in the constant region domain of IgG.<sup>1</sup> CD16 and CD32 function as low-affinity receptors for antibody-antigen immune complexes and are capable of stimulating or inhibiting cellular responses upon ligation. They can mediate effector functions such as phagocytosis, antibody-dependent cytotoxicity, and release of inflammatory mediators. CD16 and CD32 are expressed predominantly on cells of hematopoietic lineages including B cells, monocytes/macrophages, NK cells, granulocytes, mast cells, and dendritic cells. CD16 and CD32 are also found on hematopoietic progenitor cells where they appear to play a regulatory role in T and B cell development.<sup>1,2</sup> The clone 93 monoclonal antibody blocks the binding of the Fc region of immunoglobulins to its receptor (FcR).

### Product applications

- Identification and enumeration of CD16/CD32<sup>+</sup> (FcR<sup>+</sup>) cells by flow cytometry or fluorescence microscopy.

## 1.2 Recommended antibody dilution

For antibody labeling of mouse cells.

CD16/32 conjugate	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10<sup>7</sup> cells/100 µL of buffer.  
b) For optimal results, cells must be stained prior to fixation.

## 1.3 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 (4–8 °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 calf serum. Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD16/32-Biotin.
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

## 2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling 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).

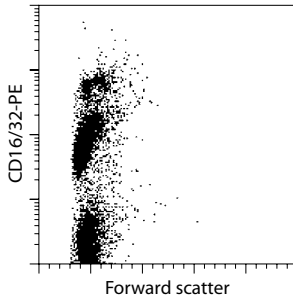
1. Resuspend up to 10<sup>7</sup> nucleated cells per 100 µL of buffer.
2. Add 10 µL of the CD16/32 antibody.
3. Mix well and refrigerate for 10 minutes in the dark (4–8 °C).  
▲ Note: Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
5. (Optional) If CD16/32-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC #130-090-857, Anti-Biotin-PE #130-090-756, or Anti-Biotin-APC #130-090-856), and continue as described in steps 3 and 4.
6. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.



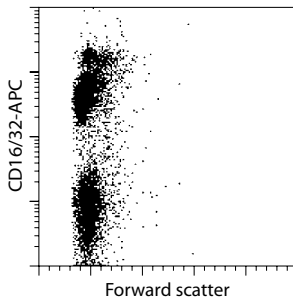
### 3. Examples of immunofluorescent staining with CD16/32 antibodies

Balb/c mouse spleen cells were stained with CD16/32 antibodies conjugated to PE (a) or APC (b) and analyzed by flow cytometry. Cells stained with CD16/32-Biotin (c) were also stained with Anti-Biotin-PE (# 130-090-756). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

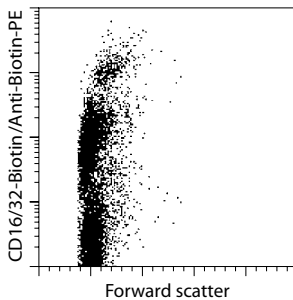
(a) Balb/c mouse spleen cells stained with CD16/32-PE.



(b) Balb/c mouse spleen cells stained with CD16/32-APC.



(c) Balb/c mouse spleen cells stained with CD16/32-Biotin and Anti-Biotin-PE.



### 4. References

1. de Andres, B. *et al.* (1998) A regulatory role for Fcγ receptors CD16 and CD32 in the development of murine B cells. *Blood* 92: 2823–2829.
2. Lynch, RG. (2000) Regulatory roles for Fcγ<sub>3</sub> (CD16) and Fcγ<sub>2</sub> (CD32) in the development of T- and B-lineage lymphoid cells. *J. Leukoc. Biol.* 67: 279–284.

#### 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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