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### 1. Description

<b>Components</b>	1 mL monoclonal CD200 antibodies, human conjugated to:						
	<table border="0"> <tr> <td>PE</td> <td>130-096-819</td> </tr> <tr> <td>APC</td> <td>130-096-816</td> </tr> <tr> <td>Biotin</td> <td>130-096-817</td> </tr> </table>	PE	130-096-819	APC	130-096-816	Biotin	130-096-817
PE	130-096-819						
APC	130-096-816						
Biotin	130-096-817						
<b>Clone</b>	OX-104 (isotype: mouse IgG1).						
<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

Clone OX-104 reacts with the human CD200 antigen, also known as MRC, MOX1/2, and OX-2, a type-1 membrane glycoprotein, which contains two immunoglobulin domains. Studies of the related gene in mouse and rat suggest that it may regulate myeloid cell activity and delivers an inhibitory signal for the macrophage lineage in diverse tissues<sup>1</sup>. CD200 may serve as a cancer stem cell marker as co-expression with other reported stem cell markers was found on prostate, breast, brain, and colon cancers<sup>2</sup>. In addition, CD200 expression was shown to be a prognostic factor in AML<sup>3</sup> and multiple myeloma<sup>4</sup>.

#### 1.2 Applications

- Identification and enumeration of CD200<sup>+</sup> cells by flow cytometry.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD200 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> 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 human serum albumin, human 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) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD200-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

## 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).

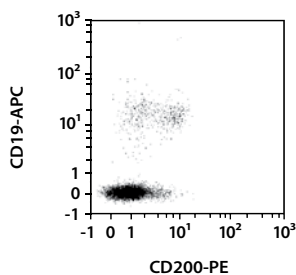
1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu$ L of buffer.
4. Add 10  $\mu$ L of the CD200 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).

▲ **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. (Optional) If CD200-Biotin was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of anti-biotin antibody, 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. Example of immunofluorescent staining with CD200 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD200 antibodies conjugated to PE as well as with CD19-APC (# 130-091-248) 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.



For more examples please refer to the respective product page at [www.miltenyibiotec.com](http://www.miltenyibiotec.com).

## 4. References

1. Minas, K. *et al.* (2006) Is the CD200/CD200 receptor interaction more than just a myeloid cell inhibitory signal? *Crit. Rev. Immunol.* 26: 213–230.
2. Kawasaki, B. T. *et al.* (2007) Co-expression of the toleragenic glycoprotein, CD200, with markers for cancer stem cells. *Biochem. Biophys. Res Commun.* 364: 778–782.
3. Tonks, A. *et al.* (2007) CD200 as a prognostic factor in acute myeloid leukaemia. *Leukemia.* 21: 566–568.
4. Moreaux, J. *et al.* (2007) CD200 is a new prognostic factor in multiple myeloma. *Blood.* 108: 4194–4197.

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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