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### 1. Description

<b>Components</b>	1 mL monoclonal CD140a antibodies, mouse conjugated to:	
	PE	130-096-271
	APC	130-096-274
	Biotin	130-096-273
<b>Clone</b>	APA5 (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

Clone APA5 reacts with the transmembrane glycoprotein CD140a, the  $\alpha$  chain of the platelet-derived growth factor receptor (PDGFR). The two isoforms of the receptor can form homodimers ( $\alpha/\alpha$  or  $\beta/\beta$ ) or heterodimers, i.e.  $\alpha/\beta$ . Receptor dimerization and transphosphorylation at tyrosine residues activates the intracellular kinase activity. CD140a has been reported to be broadly expressed in embryonic tissue, mostly in cells of mesodermal origin in adult tissue, various malignancies, and embryonic stem cell-derived cardiomyogenic cells.<sup>1-3</sup>

#### 1.2 Applications

- Identification and enumeration of CD140a<sup>+</sup> cells by flow cytometry.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD140a conjugates is **1:11 for up to 10<sup>7</sup> cells/100  $\mu$ L** of buffer for labeling of cells and subsequent analysis by flow cytometry.

The antibody is suited for staining of formaldehyd-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 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) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) as secondary antibody reagent in combination with CD140a-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

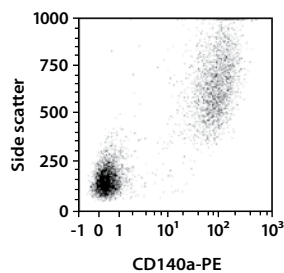
## 2. General protocol for immunofluorescent staining

▲ 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 CD140a 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 CD140a-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 CD140a antibodies

Mouse IH-3T3 cells mixed with 1881 cells were stained with CD140a antibodies conjugated to PE 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. Takakura, N. *et al.* (1997) PDGFR $\alpha$  expression during mouse embryogenesis: immunolocalization analyzed by whole-mount immunohistochemistry using the monoclonal antibody anti-mouse PDGFR alpha antibody APA5. *J. Histochem. Cytochem.* 45: 883–891.
2. Heldin, C.H. and Westermark, B. (1999) Mechanism of action and *in vivo* role of platelet-derived growth factor. *Physiol. Rev.* 79: 1283–1316.
3. Hidaka, K. *et al.* (2010) The cellular prion protein identifies bipotential cardiomyogenic progenitors. *Circ. Res.* 106: 111–119.

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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