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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.

1. Description

This product is for research use only.

Components	1 mL monoclonal CD135 antibodies, mouse conjugated to:						
	<table border="0"> <tr> <td>PE</td> <td>130-096-861</td> </tr> <tr> <td>APC</td> <td>130-096-959</td> </tr> <tr> <td>Biotin</td> <td>130-096-962</td> </tr> </table>	PE	130-096-861	APC	130-096-959	Biotin	130-096-962
PE	130-096-861						
APC	130-096-959						
Biotin	130-096-962						
Clone	A2F10 (isotype: rat 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

- Antigen: CD135
- Synonym: FLK-2/CD135
- Expression patterns: Clone A2F10 binds the 150-kDa surface protein Fms-like tyrosine kinase receptor 3 (Flt3), also known as FLK-2/CD135¹. CD135 has functional and structural homology to the class-III-receptor tyrosine kinases that include the M-CSF-, KIT-, and PDGF-receptors. The receptor is expressed on progenitor cells in adult bone marrow, thymus, brain, testis, and placenta². All hematopoietic cells develop from CD135 negative hematopoietic stem cells (HSCs) through CD135 positive progenitor cells^{3,4}. In synergy with other growth factors like G-CSF, GM-CSF, SCF, and IL-3

ligation of CD135 with Flt3 ligand promotes the proliferation and differentiation of primitive hematopoietic stem cells. As a result early B cell lineage differentiation as well as expansion of monocytes and immature dendritic cells are stimulated^{5,6}.

1.2 Applications

- Identification and enumeration of CD135⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD135 conjugates is **1:11 for up to 10^7 cells/100 μ L** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

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). 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) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD135-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.

2. General protocol for immunofluorescent staining

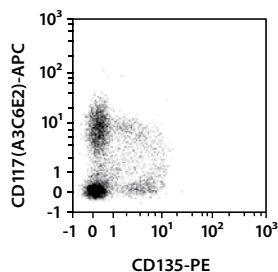
Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the CD135 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. (Optional) If CD135-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL 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 CD135 antibodies

C57BL/6J splenocytes were stained with CD135 antibodies conjugated to PE as well as with the Lineage Cell Detection Cocktail-Biotin (# 130-092-613), Anti-Biotin-FITC (# 130-090-857) and CD117-APC (130-091-729) and analyzed by flow cytometry using the MACSQuant® Analyser. Cell debris, dead cells, and lin⁺ cells were excluded.



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

1. Ogawa, M. *et al.* (1998) Flt3/Flk-2 and c-Kit are not essential for the proliferation of B lymphoid progenitor cells in the bone marrow of the adult mouse. *Exp. hematology*. 26 (6): 478–488.
2. Hannum, C. *et al.* (1994) Ligand for FLT3/FLK2 receptor tyrosine kinase regulates growth of haematopoietic stem cells and is encoded by variant RNAs. *Nature* 368 (6472): 643–648.
3. Boyer, S. W. *et al.* (2011) All Hematopoietic Cells Develop from Hematopoietic Stem Cells through Flk2/Flt3-Positive Progenitor Cells. *Cell Stem Cell* 9 (1): 64–73.
4. Böiers, C. *et al.* (2010) Expression and role of FLT3 in regulation of the earliest stage of normal granulocyte-monocyte progenitor development. *Blood* 115 (24): 5061–5068.
5. Waskow, C. *et al.* (2008) The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat. Immunol.* 9 (6): 676–683.
6. Auffray, C. *et al.* (2009) CX3CR1⁺ CD115⁺ CD135⁺ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. *J. Exp. Med.* 206 (3): 595–606.

All protocols and data sheets are available at www.miltenyibiotec.com.

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