**CD135 (FLT3) antibodies, human**

**For research use only**

One test corresponds to labeling of up to $10^6$ cells in a total volume of 100 µL.

<table>
<thead>
<tr>
<th>Product</th>
<th>Content</th>
<th>Order no.</th>
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<tbody>
<tr>
<td>CD135 (FLT3)-PE</td>
<td>for 30 tests</td>
<td>130-111-665</td>
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<tr>
<td>CD135 (FLT3)-PE</td>
<td>for 100 tests</td>
<td>130-111-587</td>
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<td>CD135 (FLT3)-APC</td>
<td>for 30 tests</td>
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<td>CD135 (FLT3)-PE-Vio770</td>
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<td>CD135 (FLT3)-VioBright 515</td>
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<td>130-111-590</td>
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<td>CD135 (FLT3)-Biotin</td>
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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.

**Technical data and background information**

**Antigen**

CD135 (FLT3)

**Clone**

REA786

**Isotype**

recombinant human IgG1

**Isotype control**

REA Control (S) antibodies

**Alternative names of antigen**

FLT3, FLK-2, STK-1

**Molecular mass of antigen [kDa]**

110

**Distribution of antigen**

hematopoietic stem cells, dendritic cells

**Product format**

Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.

**Fixation**

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

**Storage**

Store protected from light at 2–8 °C. Do not freeze.

Clone REA786 recognizes the human CD135 antigen, a single-pass type I membrane protein also known as Fms-like tyrosine kinase receptor 3 (FLT3) or fetal liver kinase-2 (FLK-2). CD135 belongs to the same family as FMS, KIT, and the two genes encoding PDGFRα and β. CD135 is normally expressed by hematopoietic stem and progenitor cells and expression is lost as hematopoietic cells differentiate. Although, CD135 expression is usually lost upon hematopoietic stem cell (HSC)
differentiation, dendritic cells are an exception, as mature dendritic cells (DCs) display persistent FLT3 expression. CD135 is also expressed on malignant hematopoietic cells including AML, ALL, and CML-BC. All hematopoietic cells develop from CD135 negative hematopoietic stem cells through CD135 positive progenitor cells. In synergy with other growth factors like G-CSF, GM-CSF, SCF, and IL-3, ligation of CD135 with FLT3 ligand promotes the differentiation, proliferation, and survival of hematopoietic progenitor cells and of dendritic cells. As a result early B cell lineage differentiation as well as expansion of monocytes and immature dendritic cells are stimulated.

Additional information: Clone REA786 displays negligible binding to Fc receptors.

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 Ca$^{2+}$ or Mg$^{2+}$ are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

Protocol for cell surface staining
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to $10^6$ cells/100 µL.
- Volumes given below are for up to $10^6$ nucleated cells. When working with fewer than $10^6$ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
  1. Determine cell number.
  2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
  3. Resuspend up to $10^6$ nucleated cells per 98 µL of buffer.
  4. Add 2 µL of the 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 biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
  8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining
Reh cells were stained with CD135 (FLT3) antibodies or with the corresponding REA Control (S) antibodies (left peak). Flow cytometry was performed using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.
References


Warranty

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