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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 Anti-Notch4 antibodies, mouse conjugated to:
	PE 130-097-117
	APC 130-097-119
	Biotin 130-097-113
Clone	HMN4-14 (isotype: hamster IgG).
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: Notch4
- Expression patterns: The HMN4-14 monoclonal antibody reacts with mouse Notch4, one of four members of the Notch family of receptors. Upon binding its membrane-bound ligand of the Delta-like or Jagged family members, the Notch receptor undergoes two proteolytic cleavage steps eventually releasing the Notch intracellular domain (NICD), which subsequently translocates into the nucleus, where it activates transcription within a multi-protein complex. Notch4 is preferentially expressed by endothelial cells, e.g., vascular smooth muscle cells and is reported to play a critical role in vasculogenesis and angiogenesis. It is also expressed on various other cell types including hematopoietic cells. In addition, the altered

expression of Notch4 by various leukemias, lymphomas, and cancers has been reported.

1.2 Applications

- Identification and enumeration of Notch4⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Notch4 conjugates is **1:11 for up to 10⁷ 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[®] 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) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with Anti-Notch4-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

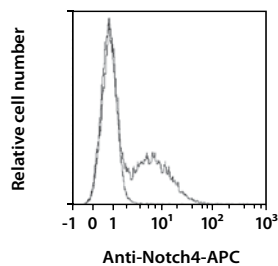
Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated 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 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-Notch4 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.

- (Optional) If Anti-Notch4-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody, and continue as described in steps 5 and 6.
- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with Anti-Notch4 antibodies

Mouse Notch4-transfected CHO cells were stained with Anti-Notch4 antibodies conjugated to APC 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/antibodies.

Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty

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