

Contents

1. Description
 - 1.1 Background Information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Example of immunofluorescent staining with Anti-Notch1 antibodies
4. References

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-Notch1 antibodies, mouse conjugated to:
	PE 130-096-554
	APC 130-096-550
	Biotin 130-096-557
Clone	22E5 (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: Notch1
- Expression patterns: The 22E5 antibody reacts with the extracellular part of the murine Notch-1 protein. Notch-1 is a 300 kDa member of the Notch family of type I transmembrane glycoproteins. Following cleavage in the Golgi, Notch forms a heterodimer composed of a 180-200 kDa disulfide-linked extracellular domain, and a 120 kDa membrane-bound intracellular portion. Upon ligand binding the latter is cleaved to form the notch intracellular domain transcription factor. Notch signaling controls cell fate decisions and is involved in multiple cellular differentiation steps during embryonic development and in adult organisms. In the immune

system, Notch-1 plays a non-redundant role for thymic T cell development¹. Notch-1 is also expressed by peripheral T cells and upregulated upon T cell activation².

1.2 Applications

- Identification and enumeration of Notch1⁺ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Notch1 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 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[®] 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-Notch1-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).

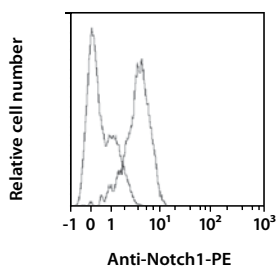
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-Notch1 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ 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 Anti-Notch1-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 Anti-Notch1 antibodies

BALB/c splenocytes were stimulated with CD3ε (# 130-092-973) and CD28 (# 130-093-375) functional grade antibodies over night and were stained with Anti-Notch1 antibodies conjugated to PE and analyzed using the MACSQuant® Analyzer. The left line in the graph shows cells from a CD4⁻ and CD8⁻ gate; the right line shows a gate on CD4⁺ and CD8⁺ cells. 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.

4. References

1. Fiorini, E. *et al.* (2009) Dynamic regulation of notch 1 and notch 2 surface expression during T cell development and activation revealed by novel monoclonal antibodies. *J. Immunol.* 183 (11): 7212–7222.
2. Radtke, F. *et al.* (2010) Notch signaling in the immune system. *Immunity* 32 (1): 14–27.

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

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, MACSQuant, and VioBlue are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2011 Miltenyi Biotec GmbH. All rights reserved.

140-009-415.01