Miltenyi Biotec

CD324 (E-Cadherin) antibodies
human

Contents

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 Monoclonal CD324 (E-Cadherin) antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
PE	130-095-413	130-099-688
APC	130-095-412	130-099-723
PE-Vio770™	130-099-141	130-099-142
APC-Vio770™	130-101-148	130-101-095

Clone 67A4 (isotype: mouse IgG1).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 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: CD324 (E-Cadherin)
- Synonym: CDH1; E-Cadherin
- Expression patterns: CD324, also known as E-Cadherin or cadherin-1, is a calcium dependent cell adhesion protein found in adherence junctions of epithelial cells, e.g., in colon, uterus, liver, keratinocytes etc. E-Cadherin also serves as a ligand for integrin alpha-E/beta-7[1]. The epitope is expressed by a variety of carcinoma-derived cell lines such as MCF-7, HT-29, T-47D, NTERA-2, and 2102Ep. Loss of E-Cadherin is thought to enable metastasis by disrupting intercellular contacts—an early step in metastatic dissemination[2]. Furthermore, E-Cadherin is a marker of undifferentiated embryonic stem (ES) cells and induced pluripotent stem (iPS) cells[3,4]. For these cells, E-Cadherin plays a major role in conveying cell survival signals[5].

1.2 Applications

- Identification and enumeration of CD324 (E-Cadherin)$^+$ cells by flow cytometry.

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD324 (E-Cadherin) conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye–conjugated antibodies to human cells, especially to monocytes.
- (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.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

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.

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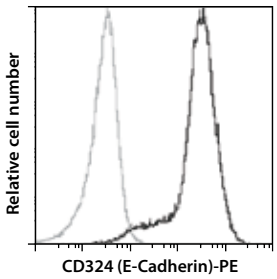
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD324 (E-Cadherin) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **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. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining with CD324 (E-Cadherin) antibodies

Human induced pluripotent stem (iPS) cells were stained with CD324 (E-Cadherin) 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. Right line represents CD324 (E-Cadherin) staining, left line represents isotype control.



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

4. References

1. Higgins J. M. *et al*. (1998). Direct and regulated interaction of integrin alphaEbeta7 with E-cadherin. J. Cell Biol. 140: 197–210.
2. Onder, T. T. *et al*. (2008). Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res. 68: 3645–3654.
3. Eastham, A. M. *et al*. (2007). Epithelial-mesenchymal transition events during human embryonic stem cell differentiation. Cancer Res. 67: 11254–11262.
4. Li, Z. *et al*. (2010) Spatially Resolved Quantification of E-Cadherin on Target hES Cells. J. Phys. Chem B. 114: 2894–2900.
5. Xu, Y. *et al*. (2010) Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc. Natl. Acad. Sci. U.S.A. 107: 5129–8134.

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

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.

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