Miltenyi Biotec

# CD44 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 CD44 antibodies, human conjugated to:

| Conjugate | Order no. 1 mL (100 tests) | Order no. 300 µL (30 tests) |
|---|---|---|
| FITC | 130-095-195 | 130-098-210 |
| PE | 130-095-180 | 130-098-108 |
| APC | 130-095-177 | 130-098-110 |
| VioBlue® | 130-099-268 | 130-099-270 |
| PE-Vio770™ | 130-099-143 | 130-099-144 |
| APC-Vio770™ | 130-099-149 | 130-099-145 |
| Biotin | 130-099-090 | 130-099-091 |

**Clone** DB105 (isotype: mouse IgG1).

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

300 µL: 30 tests or up to $3\times10^8$ 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.

Cross-reactivity: The CD44 antibody has been tested to react with

- rhesus monkey (*Macaca mulatta*) cells

### 1.1 Background information

- Antigen: CD44
- Synonym: CD44s; EMCR III; H-CAM; Pgp-1
- Expression patterns: The CD44 antibody (clone DB105) recognizes the CD44 antigen. CD44 is a marker for many types of cancer stem cells (CSC), including breast CSCs that possess higher tumorigenicity and metastatic potential[1], colorectal[2], pancreatic[3], and prostate[4,5] CSCs. In addition, expression was observed in several cancers as well as on carcinoma cell lines. Here, CD44 plays a role in cancer cell migration and matrix adhesion in response to a cellular microenvironment, thus enhancing cellular aggregation and tumor cell growth[6]. CD44 is also expressed on mesodermal cells, such as hematopoietic, fibroblastic, and glial cells.

### 1.2 Applications

- Identification and enumeration of $CD44^+$ cells by flow cytometry.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD44 conjugates is **1:11 for up to $10^7$ 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 $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- (Optional) FcR Blocking Reagent, human (# 130-059-901) 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 CD44-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.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

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## 2. General protocol for immunofluorescent staining

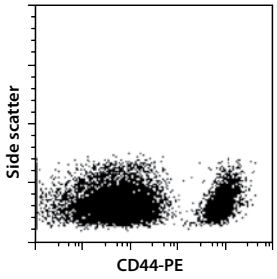
Volumes given below are for **up to $10^7$** nucleated cells. When working with fewer than $10^7$ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for $2\times10^7$ nucleated cells, use twice the volume of 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 µL of buffer.
4. Add 10 µL of the CD44 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 CD44-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 CD44 antibodies

A mixture of cells from U937 (CD44+) and 1881 (CD44–) cell lines was stained with CD44-PE antibodies 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.

## 4. References

1. Al-Hajj, M. *et al.* (2003) Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. U.S.A 100: 3983–3988.
2. Dalerba, P. *et al.* (2007) Phenotypic characterization of human colorectal cancer stem cells. Proc. Natl. Acad. Sci. U.S.A 104: 10158–10163.
3. Li, C. P. *et al.* (2007) Identification of pancreatic cancer stem cells. Cancer Res. 67: 1030–1037.
4. Patrawala, L. *et al.* (2006) Highly purified $CD44^+$ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells. Oncogene 25: 1696–1708.
5. Collins, A. T. *et al.* (2005) Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 65: 10946–10951.
6. Aruffo, A. *et al.* (1990) CD44 is the principal cell surface receptor for hyaluronate. Cell 61: 1303–1313.

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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