

CD44 antibodies

human

CD44-FITC	130-095-195
CD44-PE	130-095-180
CD44-APC	130-095-177

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1. Description

Components	1 mL CD44 antibodies, human: monoclonal CD44 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or allophycocyanin (APC).
Clone	DB105 (isotype: mouse IgG1).
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

The CD44 (clone DB105) antibody reacts with the approximately 85–95 kDa cell surface glycoprotein CD44 (also known as IN; LHR; MC56; ECMR-III). It functions as a receptor for hyaluronic acid (HA) and mediates cell-cell and cell-matrix interactions through its affinity for HA¹, and possibly also through its affinity for other ligands such as osteopontin, collagens, and matrix metalloproteinases (MMPs).

The monoclonal CD44 antibody recognizes the human and rhesus monkey (*Macaca mulatta*) CD44 antigen. In humans, CD44 is strongly expressed on mesodermal cells such as hematopoietic, fibroblastic, and glial cells. In addition an expression was observed in several cancers as well as on carcinoma cell lines. Here, it plays a role in cancer cell migration and matrix adhesion in response to a cellular microenvironment, thus enhancing cellular aggregation and tumor cell growth¹. Furthermore, CD44 was identified as a marker for cancer stem cells (CSC) including breast CSC, which possessed higher tumorigenicity and metastatic potential², colorectal CSC³, pancreatic CSC⁴, and prostate CSC^{5, 6}.

1.2 Applications

- Identification and enumeration of CD44⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human or rhesus monkey CD44⁺ cells can be isolated by using CD44 MicroBeads, human (# 130-095-194).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD44 conjugate	FITC	PE	APC
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11
- CD44 MicroBead-labeled cells	1:11	1:11	1:11
Immunohistochemistry^c			
a) The indicated antibody dilutions are for up to 10 ⁷ cells/100 µL of buffer. b) For optimal results, cells must be stained after fixation. c) The optimal antibody dilution should be determined by the user.			

- Cross-reactivity: The CD44 antibody is tested to react with rhesus monkey (*Macaca mulatta*) 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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), or Mouse IgG1-APC (# 130-092-214) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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).

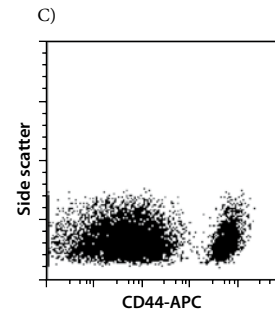
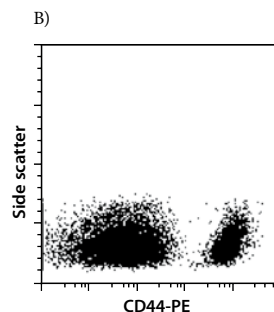
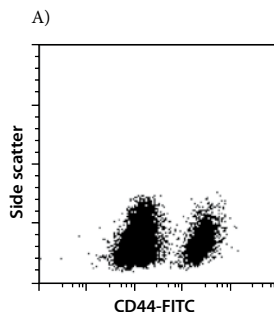
1. Determine cell number.
2. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD44 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ($2-8^\circ\text{C}$).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ 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. Examples of immunofluorescent staining with CD44 antibodies

A mixture of cells from U937 (CD44^+) and 1881 (CD44^-) cell lines were stained with CD44 antibodies conjugated to FITC (A), PE (B), or APC (C) 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.



4. References

1. Aruffo, A. *et al.* (1990) CD44 is the principal cell surface receptor for hyaluronate. *Cell* 61: 1303–1313.
2. Al-Hajj, M. *et al.* (2003) Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci.* 100: 3983–3988.
3. Dalerba, P. *et al.* (2007) Phenotypic characterization of human colorectal cancer stem cells. *Proc. Natl. Acad. Sci.* 104: 10158–10163.
4. Li, C. P. *et al.* (2007) Identification of pancreatic cancer stem cells. *Cancer Res.* 67: 1030–1037.
5. 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.
6. Collins, A. T. *et al.* (2005) Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* 65: 10946–10951.

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

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.

Warranty

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