Miltenyi Biotec

CD24 antibodies
human

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1. Description

Components	1 mL monoclonal CD24 antibodies, human conjugated to various dyes. FITC 130-095-952 PE 130-095-953 APC 130-095-954
Clone	32D12 (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 CD24 antibody reacts with the cell surface protein CD24. The human CD24 antigen is also known as cluster of differentiation 24 or heat stable antigen (HSA). The encoded sialoglycoprotein is anchored via a glycosyl phosphatidylinositol (GPI) link to the cell surface and functions as cell adhesion molecule. CD24 is a negative marker for breast cancer stem cells[1] and a positive marker for ovarian cancer stem cells[2].

1.2 Applications

- Identification and enumeration of CD24$^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human CD24$^+$ cells can be isolated or depleted by using the CD24 MicroBead Kit, human (# 130-095-951).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD24 conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells.

Cells should be stained after fixation, if formaldehyde is used as a fixative.

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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) CD44-FITC (# 130-095-195), CD44-PE (# 130-095-180), or CD44-APC (# 130-095-177). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Mouse IgG1 isotype control antibodies conjugated to, e.g., PE (# 130-092-212). For more information about isotype control antibodies refer to www. miltenyibiotec.com.
- (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.
- (Optional) gentleMACS™ Dissociator (# 130-093-235) for tissue dissociation when working with solid tissue.

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).

When working with solid tissue prepare a single-cell suspension using manual methods or the gentleMACS Dissociator.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

1. Determine cell number.

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Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

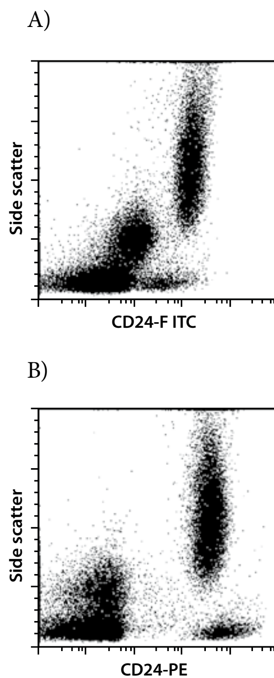
Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

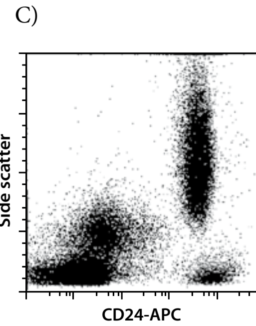
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD24 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).

   ▲ **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. Examples of immunofluorescent staining with CD24 antibodies

Human peripheral blood cells were stained with CD24 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.





For more information on cancer stem cell research please refer to www.miltenyibiotec.com/csc.

4. References

1. Al-Hajj, M. *et al.* (2003) Prospective identification of tumorigenic breast cancer cells. Proc. Natl. Acad. Sci. USA. 100: 3983–3988.
2. Gao, M. Q. *et al.* (2010) $CD24^+$ cells from hierarchically organized ovarian cancer are enriched in cancer stem cells. Oncogene 29: 2672–2680.

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.

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