CD326 (EpCAM) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
CD326 (EpCAM)-FITC	for 30 tests	130-098-113
CD326 (EpCAM)-FITC	for 100 tests	130-080-301
CD326 (EpCAM)-PE	for 30 tests	130-098-115
CD326 (EpCAM)-PE	for 100 tests	130-091-253
CD326 (EpCAM)-APC	for 30 tests	130-098-118
CD326 (EpCAM)-APC	for 100 tests	130-091-254
CD326 (EpCAM)-VioBlue	for 30 tests	130-098-092
CD326 (EpCAM)-VioBlue	for 100 tests	130-097-324
CD326 (EpCAM)-PE-Vio615	for 30 tests	130-108-386
CD326 (EpCAM)-PE-Vio615	for 100 tests	130-108-357
CD326 (EpCAM)-PE-Vio770	for 30 tests	130-099-742
CD326 (EpCAM)-PE-Vio770	for 100 tests	130-099-740
CD326 (EpCAM)-APC-Vio770	for 30 tests	130-101-159
CD326 (EpCAM)-APC-Vio770	for 100 tests	130-101-161
CD326 (EpCAM)-Biotin	for 30 tests	130-098-790
CD326 (EpCAM)-Biotin	for 100 tests	130-098-793

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.

Technical data and background information

Antigen	CD326 (EpCAM)
Clone	HEA-125
Isotype	mouse IgG1
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	EGP40, Ep-CAM, KSA, MK-1, TROP1
Molecular mass of antigen [kDa]	33
Distribution of antigen	cancer stem cells, epithelial cells, lung, ES and iPS cells
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone HEA-125 recognizes CD326, also known as human epithelial antigen (HEA), epithelial cell adhesion molecule (EpCAM), or epithelial-specific antigen (ESA) and is involved in cell adhesion. The CD326 antigen is broadly expressed on the basolateral surface of carcinoma and epithelial cells in tissues or on circulating tumor cells, and cancer stem cells, but is not found on melanoma, neuroblastoma, sarcoma, lymphoma, leukemia cells, or normal fibroblasts.

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) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated 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.

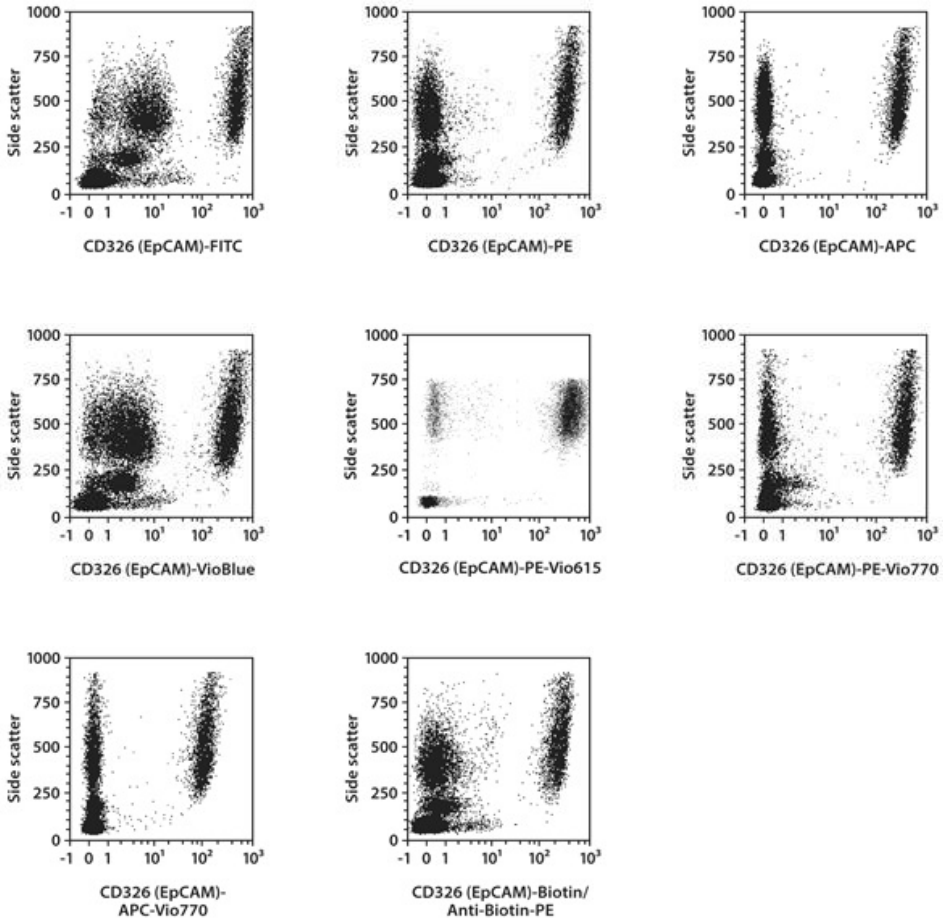
Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- 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.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the 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 biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated 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.

Examples of immunofluorescent staining

Peripheral blood leukocytes mixed with cells from a breast cancer cell line (SK-BR-3) were stained with CD326 (EpCAM) antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. FcR Blocking Reagent was added to block unwanted binding of antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals andpropidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem-conjugates.



References

1. **Moldenhauer, G. *et al.*** (1987) Epithelium specific surface glycoprotein of Mr 34,000 is a widely distributed human carcinoma marker. Br. J. Cancer 56: 714–721.
2. **Metsuyanim, S. *et al.*** (2009) Expression of stem cell markers in the human fetal kidney. PLoS One 4(68): e6709.
3. **Sheridan, C. *et al.*** (2006) $CD44^{+}/CD24^{-}$ breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast Cancer Res. 8(5): R59.

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.

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 provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.