



Miltenyi Biotec

CD326 (EpCAM) antibodies, human

For research use only

One test corresponds to labeling of up to 10^6 cells in a total volume of 100 μ L

Product	Content	Order no.
CD326 (EpCAM)-PerCP-Vio700	for 30 tests	130-111-120
CD326 (EpCAM)-FITC	for 30 tests	130-111-115
CD326 (EpCAM)-FITC	for 100 tests	130-110-998
CD326 (EpCAM)-PE	for 30 tests	130-111-116
CD326 (EpCAM)-PE	for 100 tests	130-110-999
CD326 (EpCAM)-APC	for 30 tests	130-111-117
CD326 (EpCAM)-APC	for 100 tests	130-111-000
CD326 (EpCAM)-VioBlue	for 30 tests	130-111-121
CD326 (EpCAM)-VioBlue	for 100 tests	130-111-004
CD326 (EpCAM)-VioGreen	for 30 tests	130-111-122
CD326 (EpCAM)-VioGreen	for 100 tests	130-111-005
CD326 (EpCAM)-PE-Vio615	for 30 tests	130-111-123
CD326 (EpCAM)-PE-Vio615	for 100 tests	130-111-006
CD326 (EpCAM)-PE-Vio770	for 30 tests	130-111-118
CD326 (EpCAM)-PE-Vio770	for 100 tests	130-111-001
CD326 (EpCAM)-APC-Vio770	for 30 tests	130-111-119
CD326 (EpCAM)-APC-Vio770	for 100 tests	130-111-002
CD326 (EpCAM)-PerCP-Vio700	for 100 tests	130-111-003
CD326 (EpCAM)-VioBright 515	for 30 tests	130-111-124
CD326 (EpCAM)-VioBright 515	for 100 tests	130-111-007
CD326 (EpCAM)-Biotin	for 30 tests	130-111-114
CD326 (EpCAM)-Biotin	for 100 tests	130-110-997

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	REA764
Isotype	recombinant human IgG1

Isotype control	REA Control (S) antibodies
Alternative names of antigen	EGP40, Ep-CAM, KSA, TROP1, MK-1
Entrez Gene ID	4072
Molecular mass of antigen [kDa]	33
Distribution of antigen	cancer stem cells, epithelial cells, lung, ES and iPS cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA764 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. Furthermore, it is suggested, that CD326 could be used as a surface marker for human ESCs. Additional information: Clone REA764 displays negligible binding to Fc receptors.

Reagent requirements

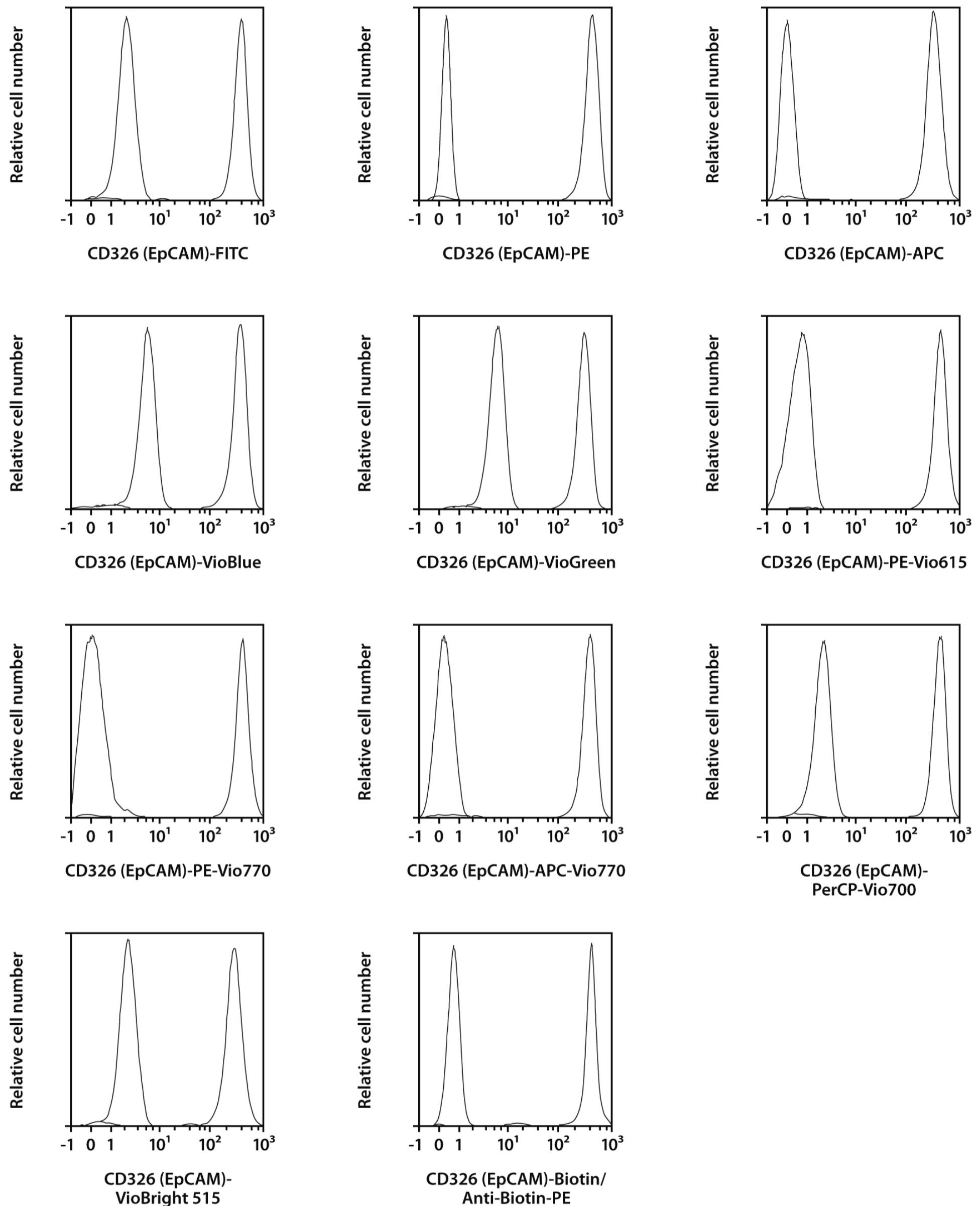
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (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 1:50 for up to 10⁶ cells/100 µL.
 - Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.
1. Determine cell number.
 2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
 3. Resuspend up to 10⁶ nucleated cells per 98 µL of buffer.
 4. Add 2 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 buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
 8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

Examples of immunofluorescent staining

SK-BR-3 cells were stained with CD326 (EpCAM) antibodies or with the corresponding REA Control (S) antibodies (left peak). Flow cytometry was performed using the MACSQuant®Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye-conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.



Warranty

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