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## 1. Description

<b>Components</b>	1 mL monoclonal CD326 (EpCAM) antibodies, human conjugated to various dyes.	
	FITC	130-080-301
	PE	130-091-253
	APC	130-091-254
	VioBlue®	130-097-324
<b>Clone</b>	HEA-125 (isotype: mouse IgG1).	
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.	
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.	
<b>Storage</b>	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 HEA antigen has been assigned to CD326 at the 8th International Workshop in Human Leukocyte Differentiation Antigens in Adelaide, Australia, in 2004. CD326, also known as EpCAM and 17-1A antigen, is a 40 kDa transmembrane glycoprotein involved in cell adhesion. The CD326 antigen is broadly expressed on the basolateral surface of carcinoma and epithelial cells, but is not found on melanoma, neuroblastoma, sarcoma, lymphoma, leukemia cells, normal fibroblasts, or hematopoietic cells.

### 1.2 Applications

- Identification and enumeration of CD326 (EpCAM)<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy.

### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD326 (EpCAM)<sup>+</sup> conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For CD326 (EpCAM) MicroBead-labeled cells use the same dilution.

The antibody is suited for staining of formaldehyd-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). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (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](http://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.

## 2. General protocol for immunofluorescent staining

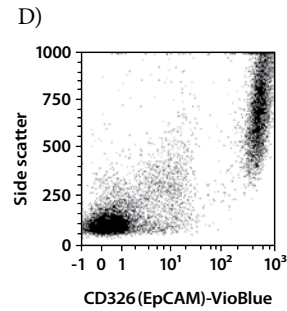
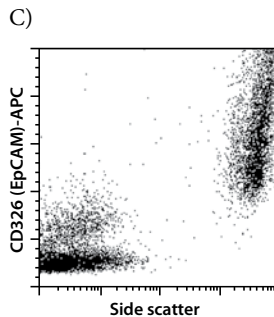
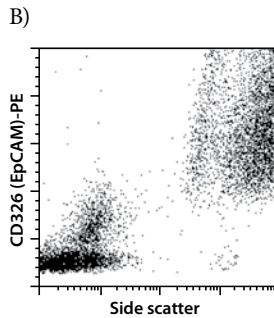
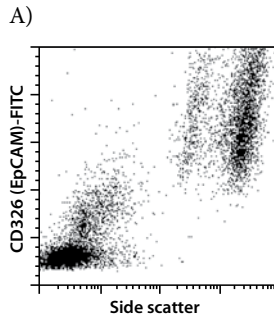
▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10<sup>7</sup> nucleated cells, use twice 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<sup>7</sup> nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD326 (EpCAM) 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.

- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

### 3. Examples of immunofluorescent staining with CD326 (EpCAM) antibodies

Peripheral blood leukocytes mixed with cells from a breast cancer cell line (SK-BR-3) were stained with CD326 (EpCAM) antibodies conjugated to FITC (A), PE (B), APC (C), or VioBlue (D) and analyzed by flow cytometry. FcR Blocking Reagent (# 130-059-901) was added to block unwanted binding of antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



### 4. References

- 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.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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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