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### 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.

### 1. Description

**This product is for research use only.**

**Components** Monoclonal CD105 antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
FITC	130-098-774	130-098-778
PE	130-094-941	130-098-906
APC	130-094-926	130-099-125
VioBlue®	130-099-666	130-099-667
PE-Vio770™	130-099-889	130-099-890
Biotin	130-094-916	–

**Clone** 43A4E1 (isotype: mouse IgG1).

**Capacity** 1 mL: 100 tests or up to 10<sup>9</sup> total cells  
300 µL: 30 tests or up to 3×10<sup>8</sup> 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

- Antigen: CD105
- Synonym: Endoglin

- **Expression patterns:** The CD105 antigen, also known as endoglin, serves as a receptor for the growth and differentiation factors TGF-β1 and TGF-β3. An epitope of CD105 is recognized by the SH-2 antibody<sup>1</sup>, which was raised against human mesenchymal stromal cells (MSC) that show mesodermal differentiation capacity.<sup>2</sup> Therefore, it can be used for studies on mesengensis. CD105 is also expressed on mature endothelial cells and on some leukemic cells of B lymphoid and myeloid origin.

#### 1.2 Applications

- Identification and enumeration of CD105<sup>+</sup> cells by flow cytometry.

#### 1.3 Recommended antibody dilution

The recommended antibody dilution for all CD105 conjugates is **1:11 for up to 10<sup>7</sup> cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

Cells should be stained prior to 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). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.

- (Optional) FcR Blocking Reagent, human (#130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Tandem Signal Enhancer, human (#130-099-888) to reduce non-specific binding of tandem dye-conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (#130-090-756) as secondary antibody reagent in combination with CD105-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/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.

## 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  $2 \times 10^7$  nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

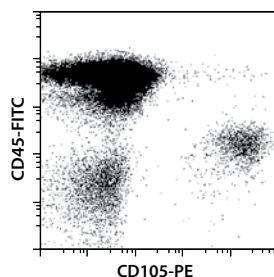
1. Determine cell number.
2. Centrifuge cell suspension at  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the CD105 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator ( $2-8^\circ\text{C}$ ).

▲ **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  $300 \times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD105-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of 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.

## 3. Example of immunofluorescent staining with CD105 antibodies

Human peripheral blood mononuclear cells (PBMCs) were spiked with human umbilical vein endothelial cells (HUVECs) and stained with CD105 antibodies conjugated to PE well as with CD45-FITC (# 130-080-202) 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 examples please refer to the respective product page at [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).

## 4. References

1. Barry, F. P. *et al.* (1999) The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem. Biophys. Res. Commun.* 265: 134–139.
2. Majumdar, M. K. *et al.* (2000) Isolation, characterization, and chondrogenic potential of human bone marrow-derived multipotential stromal cells. *J. Cell. Physiol.* 185: 98–106.

Refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com) for all data sheets and protocols.

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