

# CD309 (VEGFR-2/KDR) antibodies

## human

CD309 (VEGFR-2/KDR)-PE	130-093-598
CD309 (VEGFR-2/KDR)-APC	130-093-601
CD309 (VEGFR-2/KDR)-Biotin	130-093-603
CD309 (VEGFR-2/KDR) pure	130-095-324

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### 1. Description

<b>Components</b>	1 mL CD309 (VEGFR-2/KDR) antibodies, human: monoclonal CD309 (VEGFR-2/KDR) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
<b>Clone</b>	ES8-20E6 (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 CD309 (VEGFR-2/KDR) antibody recognizes the CD309 antigen, also known as vascular endothelial growth factor receptor-2 (VEGFR-2) and kinase insert domain-containing receptor (KDR). CD309 is a high-affinity receptor for VEGF and plays a role in hematopoiesis. It is also involved in angiogenesis, both during embryogenesis and in the context of homeostatic or pathological events, e.g., vascularization of tumors. It has been identified on a subset of hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs) as well as on their common progenitor, the hemangioblast.<sup>1–3</sup> CD309 expression is also found on mature endothelial cells (ECs).<sup>4</sup>

#### 1.2 Applications

- Identification and enumeration of CD309<sup>+</sup> cells in peripheral blood, bone marrow, or other tissues cells by flow cytometry or fluorescence microscopy.

#### 1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD309 (VEGFR-2/KDR) conjugate	PE	APC	Biotin
Flow cytometry <sup>a</sup>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11

- a) The indicated antibody dilutions are for up to 10<sup>7</sup> cells/100 µL of buffer.  
 b) For optimal results, cells must be stained prior to fixation.

#### 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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-VioBlue® (# 130-094-669), Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD309 (VEGFR-2/KDR)-Biotin.
- (Optional) Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485) for subsequent indirect magnetic labeling.
- (Optional) CD34-FITC (# 130-081-001), CD45-FITC (# 130-080-202), or CD133/1 (AC133)-PE (# 130-080-801). For more information about antibodies refer to [www.miltenyibiotec.com](http://www.miltenyibiotec.com).
- (Optional) Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) for isotype control.
- (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.

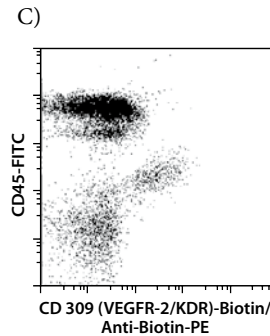
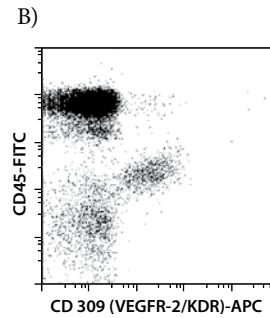
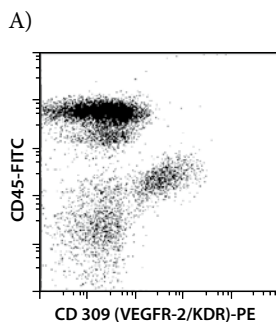
## 2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to  $10^7$  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 CD309 (VEGFR-2/KDR) 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 CD309 (VEGFR-2/KDR)-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  of anti-biotin antibody (Anti-Biotin-VioBlue, Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), 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. Examples of immunofluorescent staining with CD309 (VEGFR-2/KDR) antibodies

Human peripheral blood mononuclear cells (PBMCs) were spiked with human umbilical vein endothelial cells (HUVECs), stained with CD309 (VEGFR-2/KDR) antibodies conjugated to PE (A) or APC (B) as well as with CD45-FITC (# 130-080-202) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD309 (VEGFR-2/KDR)-Biotin (C) were stained with Anti-Biotin-APC (# 130-090-856) as well as CD45-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## 4. References

1. Auerbach R. *et al.* (1996) Hematopoietic stem cells in the mouse embryonic yolk sac. *Stem Cells* 14: 269–280.
2. Eichmann A. *et al.* (1997) Ligand-dependent development of the endothelial and hemopoietic lineages from embryonic mesodermal cells expressing vascular endothelial growth factor receptor 2. *Proc. Natl. Acad. Sci. USA* 94: 5141–5146.
3. Shalaby F. *et al.* (1995) Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 376: 62–66.
4. Flamme I. *et al.* (1995) Vascular endothelial growth factor (VEGF) and VEGF receptor 2 (flk-1) are expressed during vasculogenesis and vascular differentiation in the quail embryo. *Dev. Biol.* 169: 699–712.

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.

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