CD321 (JAM1) 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.
CD321 (JAM1)-FITC	for 30 tests	130-109-481
CD321 (JAM1)-FITC	for 100 tests	130-109-402
CD321 (JAM1)-PE	for 30 tests	130-109-482
CD321 (JAM1)-PE	for 100 tests	130-109-403
CD321 (JAM1)-APC	for 30 tests	130-109-483
CD321 (JAM1)-APC	for 100 tests	130-109-404
CD321 (JAM1)-VioBlue	for 30 tests	130-109-480
CD321 (JAM1)-VioBlue	for 100 tests	130-109-401
CD321 (JAM1)-PE-Vio770	for 30 tests	130-109-484
CD321 (JAM1)-PE-Vio770	for 100 tests	130-109-405
CD321 (JAM1)-APC-Vio770	for 30 tests	130-109-485
CD321 (JAM1)-APC-Vio770	for 100 tests	130-109-406
CD321 (JAM1)-PerCP-Vio700	for 30 tests	130-109-486
CD321 (JAM1)-PerCP-Vio700	for 100 tests	130-109-407
CD321 (JAM1)-Biotin	for 30 tests	130-109-479
CD321 (JAM1)-Biotin	for 100 tests	130-109-400

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	CD321 (JAM1)
Clone	REA605
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	JAM-1, JAM-A, PAM-1, F11 R
Molecular mass of antigen [kDa]	30
Distribution of antigen	red blood cells, leukocytes, platelets, endothelial cells, epithelial cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA605 recognizes the human CD321 antigen, a cell-surface adhesion molecule, also known as JAM1 or JAMA. CD321 is a type I transmembrane glycoprotein belonging to the immunoglobin superfamily. It has two extracellular Ig-like domains, a transmembrane region and a short cytosolic tail. CD321 is expressed on a variety of cell types: erythrocytes, leukocytes, platelets, as well as endothelial and epithelial cells. CD321 has different functions. It is a regulator of tight junction assembly in epithelia and acts as a receptor for reovirus. Furthermore CD321 binds integrin LFA1 and is involved in leukocyte transmigration. In addition, CD321 is a platelet receptor.
Additional information: Clone REA605 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 Ca^{2+} or Mg^{2+} 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.

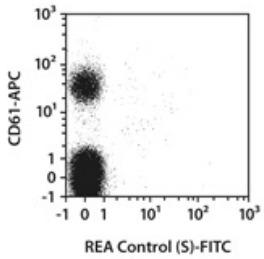
Protocol for cell surface staining

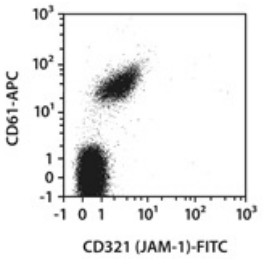
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10^7 cells/100 µL of buffer.
- Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher 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

Human platelets were stained with CD321 (JAM1) antibodies or with the corresponding REA Control (S) antibodies (left image) as well as with CD61 antibodies. 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.


CD61-APC
REA Control (S)-FITC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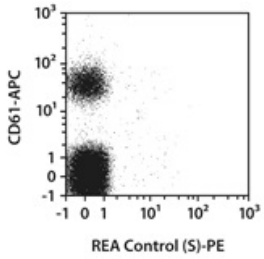
CD321 (JAM-1)-FITC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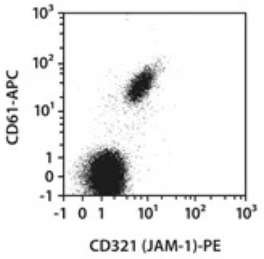
REA Control (S)-PE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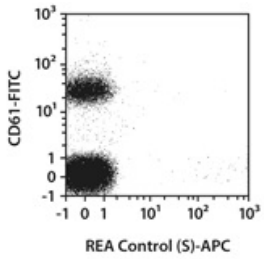
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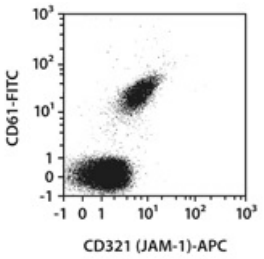
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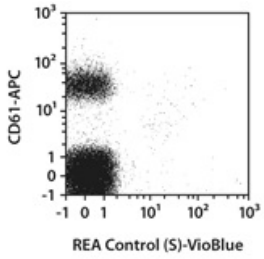
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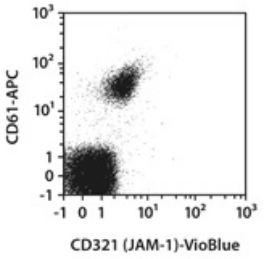
REA Control (S)-VioBlue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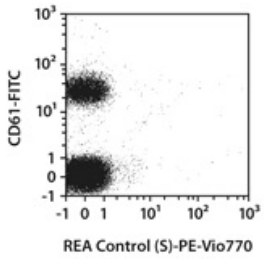
CD321 (JAM-1)-VioBlue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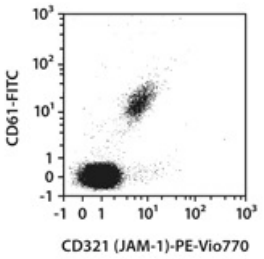
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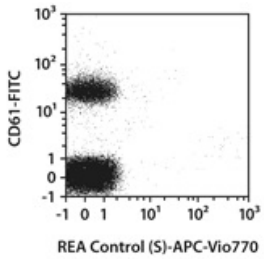
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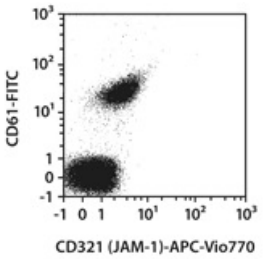
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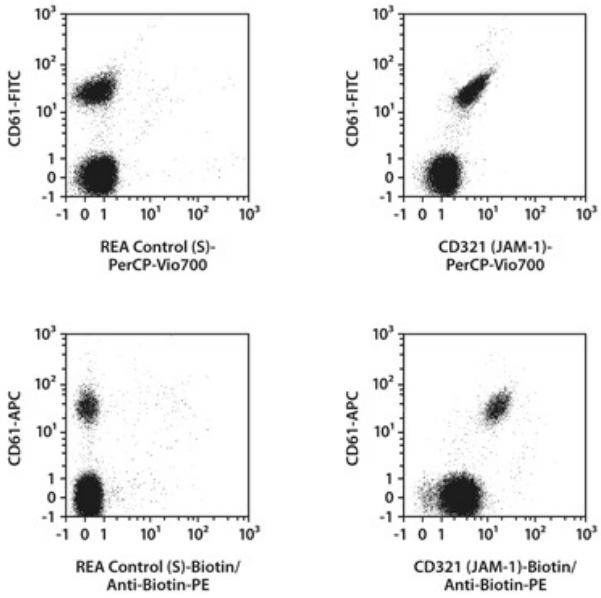

References

1. **Ozaki, H. *et al.*** (1999) Cutting edge: combined treatment of TNF-alpha and IFN-gamma causes redistribution of junctional adhesion molecule in human endothelial cells. J. Immunol. 163(2): 553–557.
2. **Sobocka, M. B. *et al.*** (2000) Cloning of the human platelet F11 receptor: a cell adhesion molecule member of the immunoglobulin superfamily involved in platelet aggregation. Blood 95(8): 2600–2609.
3. **Fraemohs, L. *et al.*** (2004) The functional interaction of the beta 2 integrin lymphocyte function-associated antigen-1 with junctional adhesion molecule-A is mediated by the I domain. J. Immunol. 173(10): 6259–6264.

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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**
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