

Anti-ESAM 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.
Anti-ESAM-Biotin	for 100 tests	130-115-037
Anti-ESAM-FITC	for 30 tests	130-115-080
Anti-ESAM-FITC	for 100 tests	130-115-038
Anti-ESAM-PE	for 30 tests	130-115-081
Anti-ESAM-PE	for 100 tests	130-115-039
Anti-ESAM-APC	for 30 tests	130-115-082
Anti-ESAM-APC	for 100 tests	130-115-040
Anti-ESAM-PE-Vio770	for 30 tests	130-115-083
Anti-ESAM-PE-Vio770	for 100 tests	130-115-041
Anti-ESAM-APC-Vio770	for 30 tests	130-115-084
Anti-ESAM-APC-Vio770	for 100 tests	130-115-042
Anti-ESAM-Biotin	for 30 tests	130-115-079

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	ESAM
Clone	REA901
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	Endothelial cell-selective adhesion molecule
Entrez Gene ID	90952
Molecular mass of antigen [kDa]	38
Distribution of antigen	endothelial cells, megakaryocytes, platelets
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 REA901 recognizes the human endothelial cell-selective adhesion molecule (ESAM) antigen belonging to the JAM family, which is also known as a transmembrane tight junction protein composed of a single transmembrane domain and a cytoplasmic domain. ESAM is found in a high level on endothelial cells, on activated platelets, and megakaryocytes.

Additional information: Clone REA901 displays negligible binding to Fc receptors.

Reagent requirements

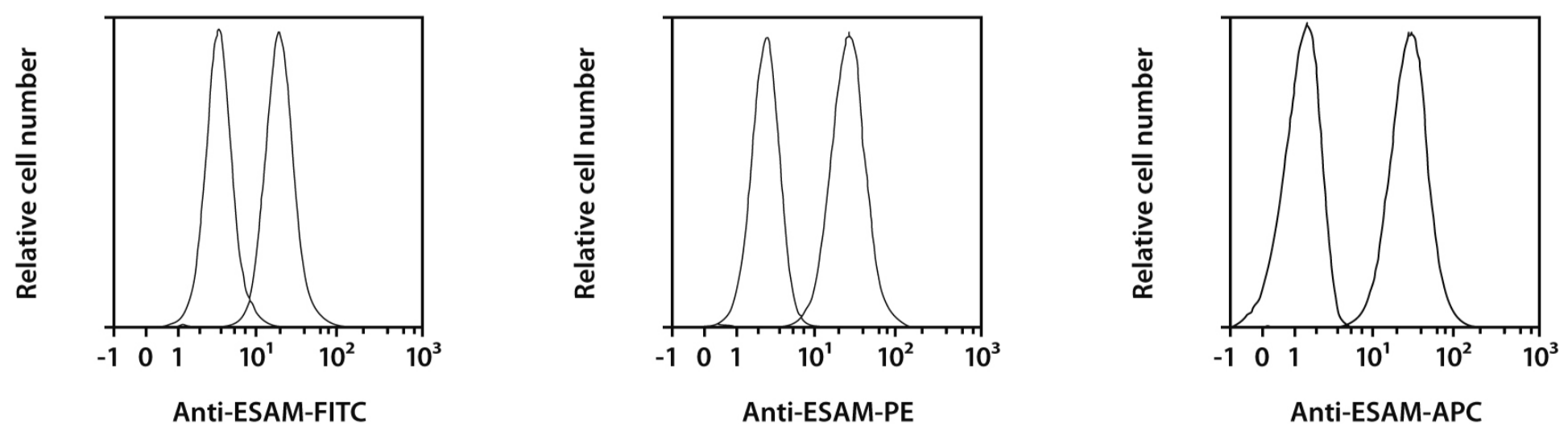
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other 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.

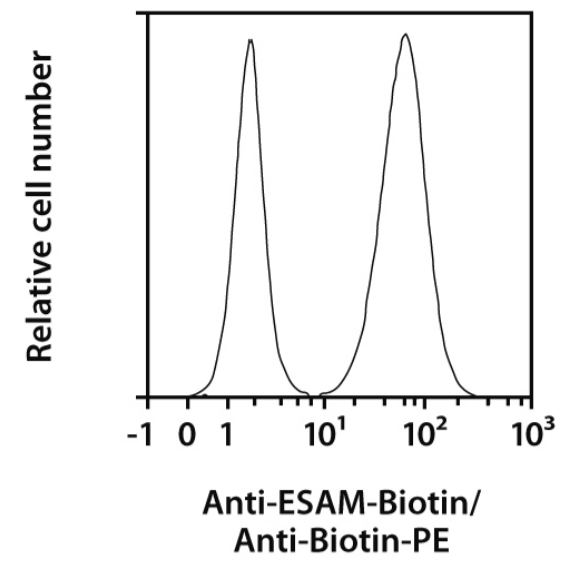
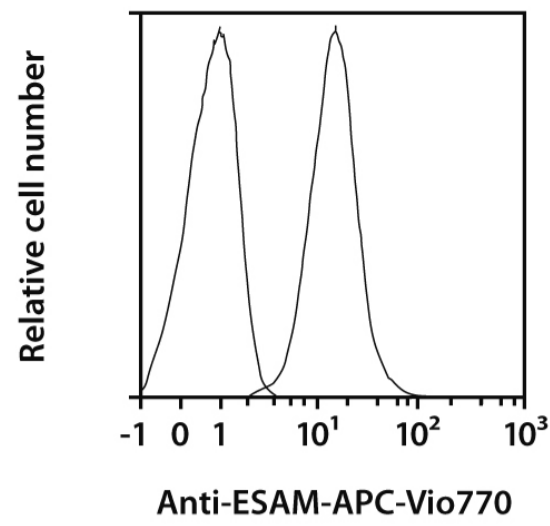
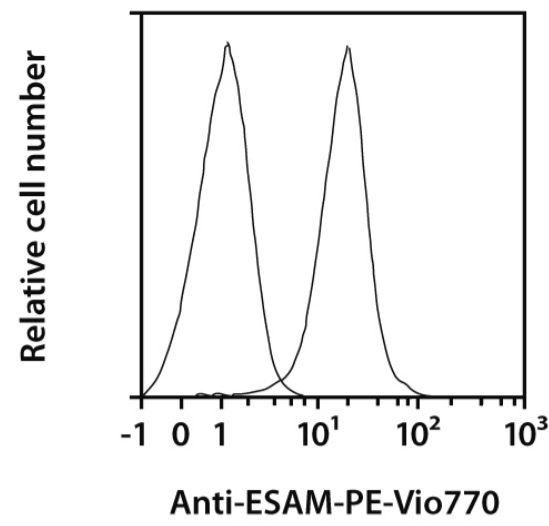
Protocol for cell surface staining

- 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

Human umbilical vein endothelial cells (HUVEC) were stained with Anti-ESAM 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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