

ORDERING INFORMATION

Catalog Number: MAB4204

Clone: 408519

Lot Number: ZFP02

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human ESAM

Immunogen: NS0-derived rhESAM

Ig class: mouse IgG_{2b}

Recommended Applications:

Flow cytometry
Immunocytochemistry

Other Application:

Direct ELISA

Background

Endothelial cell adhesion molecule (ESAM) is a 55 kDa type I transmembrane glycoprotein of the JAM family of immunoglobulin superfamily molecules. The 390 aa human ESAM contains a 216 aa extracellular domain (ECD) with a V-type and a C2-type Ig domain. The ECD of human and mouse ESAM share 69% aa identity. ESAM is expressed on endothelial cells, activated platelets and megakaryocytes. ESAM mediates endothelial cell homophilic adhesion and supports neutrophil extravasation through tight junctions.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant human ESAM (rhESAM; aa 30 - 247; Accession # NP_620411). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 0.2 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody was selected for its ability to detect human ESAM in direct ELISAs. In this application, this antibody shows no cross-reactivity with rmESAM.

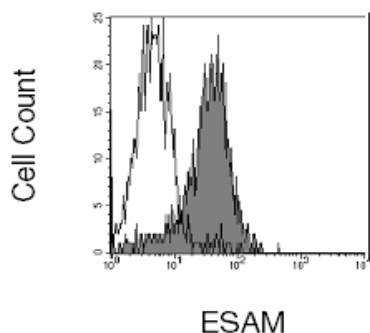
Applications

Flow cytometry - This antibody was validated for flow cytometry using HUVECs. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 2.5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled monoclonal antibodies may be visualized by adding a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome.

Immunocytochemistry - This antibody was used at a concentration of 10 µg/mL to detect ESAM in HUVECs. Cells were fixed with PBS containing 4% paraformaldehyde and blocked with PBS containing 10% normal donkey serum, 0.1% Triton® X-100, and 1% BSA. After blocking, cells were incubated with diluted primary antibody followed by NL557-coupled anti-mouse IgG Catalog # NL007 in the dark. Between each step, cells were washed with PBS containing BSA.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human ESAM. The detection limit for rhESAM is approximately 1 ng/well.

Optimal dilutions should be determined by each laboratory for each application.



HUVECs were stained with anti-ESAM (R&D Systems, Cat. # MAB4204, filled histogram) or isotype control antibody (R&D Systems, Cat. # MAB0041, open histogram), followed by PE-conjugated anti-mouse antibody (R&D Systems, Cat. # F0102B).