

Anti-human Endothelin B Receptor Antibody

ORDERING INFORMATION

Catalog Number: AF4496

Lot Number: CADY01

Size: 100 µg

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

Storage: -20° C

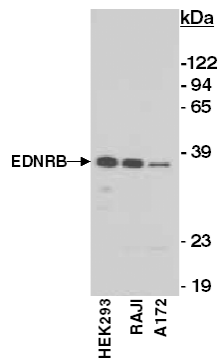
Reconstitution: sterile PBS

Specificity: human EDNRB

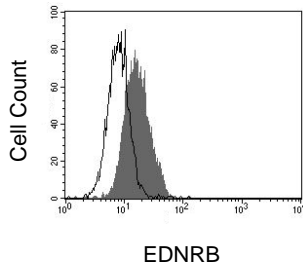
Immunogen: *E. coli*-derived rhEDNRB (aa 27 - 101)

Ig Type: sheep IgG

Applications: Western blot
Flow cytometry
Immunocytochemistry
Direct ELISA



Detection of EDNRB with AF4496. Cell lysates were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL sheep anti-hEDNRB.



A172 cells were stained with anti-EDNRB (R&D Systems, Cat. # AF4496, filled histogram), or control antibody (R&D Systems, Cat. # 5-001-A, open histogram) followed by NL557-conjugated donkey anti-sheep IgG (R&D Systems, Cat. # NL010).

Background

EDNRB (Endothelin B Receptor) is a member of the beta-family of rhodopsin receptors. It binds endothelin 1, 2 and 3, and is found on endothelial cells where it mediates vasodilation. Mature human EDNRB is a 7-transmembrane glycoprotein that is 416 amino acids (aa) in length. It contains a 75 aa N-terminal extracellular region (aa 27 - 101), and a 44 aa C-terminal cytoplasmic domain. There are three EDNRB variants that affect aa 27 - 101. One shows a 90 aa N-terminal extension, a second shows the same 90 aa N-terminal substitution coupled with a deletion of aa 268 - 398, and a third shows proteolytic cleavage between Arg64 - Ser65. Over aa 27 - 101, human EDNRB shares 67% and 97% aa identity with mouse and canine EDNRB, respectively.

Preparation

Produced in sheep immunized with purified, *E. coli*-derived, recombinant human Endothelin B Receptor (rhEDNRB; aa 27 - 101; Accession # P24530). Human EDNRB specific IgG was purified by human EDNRB 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 0.5 mL of PBS is used, the antibody concentration will be 0.2 mg/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 has been selected for its ability to recognize human EDNRB in the applications listed below.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Flow cytometry - This antibody was tested in flow cytometry using A172 cells. For intracellular staining to detect EDNRB, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 50 µg/mL and add 10 µL of the diluted solution to 1 - 5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-sheep IgG conjugated to a fluorochrome.

Immunocytochemistry - This antibody has been used at a concentration of 10 µg/mL to detect EDNRB in A172 cells. Cells were fixed with PBS containing 4% paraformaldehyde for 20 minutes at room temperature and blocked with PBS containing 10% normal donkey serum, 0.1% Triton® X-100, and 1% BSA for 45 minutes at room temperature. After blocking, cells were incubated with diluted primary antibody for 3 hours at room temperature followed by R&D Systems NorthernLights™ 557 donkey anti-sheep IgG (Catalog # NL010) at room temperature for 1 hour. Between each step, cells were washed with PBS containing 0.1% BSA.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human EDNRB. The detection limit for rhEDNRB is approximately 0.2 ng/well.

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

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475