Anti-human Endoglycan/PODLX2 Antibody

ORDERING INFORMATION

Catalog Number: AF1524

Lot Number: IWI01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human Endoglycan

Immunogen: NS0-derived rhEndoglycan (aa 33 - 500)

Ig Type: human Endoglycan specific goat IgG

Applications: Direct ELISA
Western blot
Immunohistochemistry
Flow cytometry

Preparation
Produced in goats immunized with purified, NS0-derived, recombinant human Endoglycan (rhEndoglycan; aa 33 - 500) extracellular domain. Human Endoglycan specific IgG was purified by human Endoglycan affinity chromatography. Endoglycan, also named podocalyxin-like 2 (PODLX2), is a member of the CD34 family of sialomucins. It is a Type I transmembrane protein expressed on endothelial cells, early hematopoietic progenitors and leukocyte subpopulations. Specific endoglycan glycoforms function as L-Selectin ligands.

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

Endotoxin Level
< 0.1 EU per 1 μg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 0.1 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 Endoglycan in direct ELISAs and western blots. In these formats, this antibody shows less than 5% cross-reactivity with rhPODXL.

Applications
Direct ELISA - This antibody can be used at 0.5 - 1.0 μg/mL with the appropriate secondary reagents to detect human Endoglycan. The detection limit for rhEndoglycan is approximately 0.1 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μg/mL with the appropriate secondary reagents to detect human Endoglycan. The detection limit for rhEndoglycan is approximately 0.5 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry - This antibody can be used with the appropriate secondary reagents at the concentration of 5 - 10 μg/mL in fixed cells. Cells were fixed with 4% paraformaldehyde in PBS at room temperature for 20 min. then blocked with 0.1% Triton X-100, 1% BSA and 10% normal donkey serum in PBS at room temperature for 45 min. After blocking, cells were incubated with the diluted primary antibody overnight at 4° C followed by Rhodamine Red coupled anti-goat IgG antibody or another appropriate secondary antibody at room temperature in the dark for an hour. Between each step, cells were washed with 0.1% BSA in PBS.

Flow Cytometry - Dilute this antibody to 0.1 mg/mL and add 10 μL of this solution to 1 - 2.5 x 10^5 cells in a total reaction volume not exceeding 200 μL. The binding of unlabeled primary antibodies may be visualized by adding 10 μL of a 25 μg/mL stock solution of a secondary developing reagent such as goat anti-goat IgG conjugated to a fluorochrome.

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

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7/30/03