

ORDERING INFORMATION

Catalog Number: MAB23972

Clone: 295208

Lot Number: XOO01

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human CRISP-3

Immunogen: NS0-derived rhCRISP-3

Ig class: mouse IgG_{2b}

Recommended Application:
Flow cytometry

Other Applications:
Western blot
Direct ELISA

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 Cysteine-Rich Secretory Protein 3 (rhCRISP-3; aa 21 - 245; Accession # NP_006052). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. CRISP-3 belongs to the large CRISP family of secreted proteins with a characteristic C-terminal cysteine-rich domain. CRISP-3 is expressed in salivary glands, prostate, pancreas, and in the secretory granules of neutrophilic granulocytes. In plasma, CRISP-3 circulates in a complex with alpha (1) B-glycoprotein, a member of the Ig superfamily.

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 detects human CRISP-3 in direct ELISA and western blots. In these applications this antibody shows no cross-reactivity with rhCRISP-2.

Applications

Flow Cytometry –This antibody has been validated for flow cytometry using PC-3 cells. For intracellular staining to detect human CRISP-3, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 25 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. Following a 30 minute incubation, cells should be washed with 0.1% saponin prior to addition of a secondary developing reagent. The binding of unlabeled monoclonal antibodies may be visualized by adding 10 µL of a 25 µg/mL solution of a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome. Cells should be washed for a final time in 0.1% saponin prior to flow cytometric analysis.

Western Blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human CRISP-3. Using a colorimetric detection system, the detection limit for rhCRISP-3 is approximately 25 ng/lane under non-reducing conditions. Use of this antibody under reducing conditions is not recommended. Chemiluminescent detection with WesternGlo Chemiluminescent Detection Substrate (R&D Systems, Catalog # AR004) will increase sensitivity by 5 to 50 fold. In this application, the use of anti-CRISP-3 monoclonal antibody, R&D Systems, Catalog # MAB2397, is recommended.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human CRISP-3. The detection limit for rhCRISP-3 is approximately 20 ng/well.

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