



Monoclonal Anti-human HCRTR2 Antibody

ORDERING INFORMATION

Catalog Number: MAB5246

Clone: 456738

Lot Number: CANZ02

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

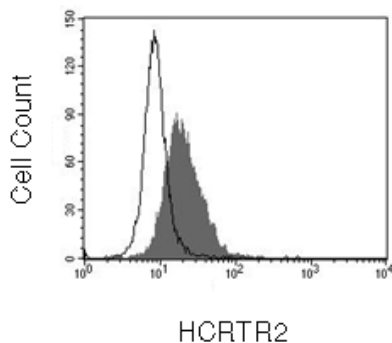
Specificity: human HCRTR2

Immunogen: human HCRTR2-transfected NS0 cells

Ig class: mouse IgG_{2A}

Recommended Applications:

Flow cytometry
Immunocytochemistry



A172 cells were stained with anti-HCRTR2 (R&D Systems, Cat. # MAB5246, filled histogram) or isotype control (R&D Systems, Cat. # MAB003, open histogram) followed by PE-conjugated anti-mouse antibody (R&D Systems, Cat. # F0102B).

Background

Hypocretin receptor 2 (HCRTR2); also known as orexin receptor 2 or OX2R) is a 40 kDa 7-transmembrane G-protein-coupled glycoprotein that is a high affinity receptor for orexins A and B (hypocretins 1 and 2). In mouse brain, engagement of HCRTRs promotes wakefulness, such that absence of either orexins or their receptors creates a narcolepsy-like state. It also influences reward circuits involving food or addictive drugs. The extracellular portions of human HCRTR2 share 92 and 93% aa identity with corresponding portions of mouse and rat HCRTR2, respectively.

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 human HCRTR2 transfected NS0 cells (rhHCRTR2; Accession # O43614). 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 detects human HCRTR2-transfected cells but not irrelevant transfectants by flow cytometry.

Applications

Flow cytometry - This antibody was tested for flow cytometry using A172 cells. For intracellular staining to detect HCRTR2, 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. The binding of unlabeled monoclonal antibodies may be visualized by adding a secondary developing reagent such as anti-mouse IgG conjugated to a fluorochrome.

Immunocytochemistry - This antibody was used at a concentration of 10 µg/mL to detect HCRTR2 in A172 cells. 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.

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

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