

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human NPY1R: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 556153

Isotype: mouse IgG_{2b}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

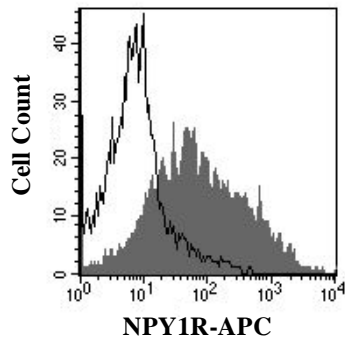
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing NPY1R within a population and qualitatively determine the density of NPY1R on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with NS0 cells transfected with human NPY1R (Accession # P25929). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of NPY1R is determined by flow cytometry using 620-650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660-670 nm.



MCF-7 cells were stained with APC-conjugated anti-human NPY1R (Catalog # FAB6400A; filled histogram) or APC-conjugated isotype control (Catalog # IC0041A; open histogram).

Background Information

Neuropeptide Y receptor type 1 (NPY1R) is a 44 kDa (unglycosylated) member of the G protein coupled receptor 1 family. Human NPY1R is 384 amino acids in length and contains seven transmembrane regions and three potential sites for N-linked glycosylation. In addition, amino acid 338 is an S-palmitoyl cysteine lipid-binding site and amino acid 368 is a phosphoserine. Human NPY1R shares 94% and 93% amino acid sequence identity with mouse and rat NPY1R, respectively. Functionally, NPY1R is a receptor for neuropeptide Y and peptide YY.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using MCF-7 cells.

- Cells may be Fc-blocked with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 10 µL of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
- Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
- The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse IgG_{2b} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.