

## Reagents Provided

**Allophycocyanin (APC)-conjugated goat polyclonal anti-human/mouse CELSR2:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Isotype:** goat IgG

## Reagents Not Provided

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

## Storage

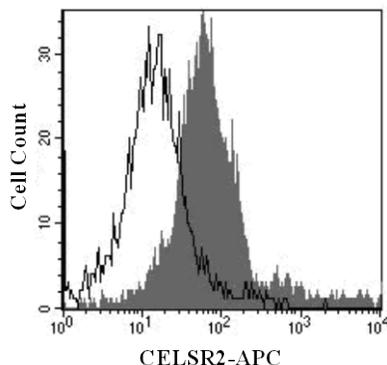
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing CELSR2 within a population and qualitatively determine the density of CELSR2 on cell surfaces by flow cytometry.

## Product Description

This antibody was produced in goats immunized with purified, *E. coli*-derived recombinant human CELSR2 (rhCELSR2; Cys51-Phe231; Accession # Q9HCU4). CELSR2 specific IgG was purified by human CELSR2 affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of CELSR2 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.



SHSY5Y cells were stained with APC-conjugated anti-human CELSR2 (Catalog # FAB6739A, filled histogram) or isotype control (Catalog # IC108A, open histogram).

## Background Information

CELSR2 (Cadherin EGF LAG seven-pass G-type receptor 2; also known as cadherin family member 10/CDHF10, Flamingo1, and EGFL2) is a 300-330 kDa member of the LN-7TM subfamily, GPCR 2 family of proteins. It is expressed on neurons, breast epithelium, Sertoli cells, and germ cells, and through homophilic interactions, serves as either an adhesion or guidance molecule. Mature human CELSR2 is 2892 amino acids (aa) in length (aa 32-2923). It is a highly complex 7-transmembrane protein that contains a 2349 aa extended N-terminal extracellular region (aa 32-2380) plus a 310 aa C-terminal cytoplasmic domain. The N-terminal region contains nine consecutive cadherin domains (aa 182-1146), followed by a mixture of seven EGF-like and three laminin-like domains. There is a proteolytic cleavage site between Met2356 and Thr2357 that generates a 250 kDa soluble fragment and a (mature) 60-65 kDa transmembrane segment that may reside on the cell membrane. Over aa 51-231, human CELSR2 shares 93% aa identity with mouse CELSR2.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using the SHSY5Y cell line.

- Cells may be Fc-blocked with 1 µg of human IgG/10<sup>5</sup> 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 10<sup>6</sup> 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 analysis, cells in a separate tube should be treated with APC-labeled goat IgG 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.