

## Reagents Provided

### Phycoerythrin (PE)-conjugated goat polyclonal anti-mouse EphB3:

Supplied as 50 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

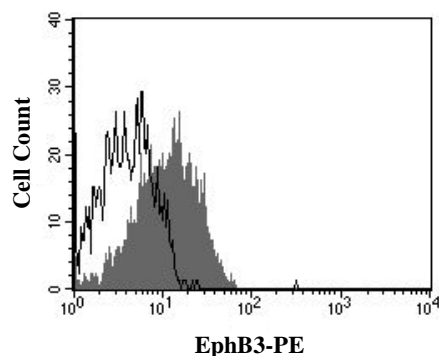
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing EphB3 within a population and qualitatively determine the density of EphB3 on cell surfaces by flow cytometry.

## Product Description

This antibody was produced in goats immunized with purified, NS0-derived, recombinant mouse EphB3 (rmEphB3) extracellular domain. EphB3 specific IgG was purified by mouse EphB3 affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of EphB3 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.



COLO 205 cells were stained with PE-conjugated anti-mouse EphB3 (Catalog # FAB432P, filled histogram) or PE-conjugated isotype control (Catalog # IC108P, open histogram).

## Background Information

EphB3, also known as Cek10, Tyro6, Sek4, Hek2, and Mdk5, is a 130 kDa member of the transmembrane Eph receptor tyrosine kinase family. The A and B classes of Eph proteins are distinguished by Ephrin ligand binding preference, but have a common structural organization. Eph-Ephrin interactions are widely involved in the regulation of cell migration, tissue morphogenesis, and cancer progression. EphB3 is widely expressed during development and in the adult it shows a complementary tissue distribution to the Ephrin-B ligand. EphB3 function is important in vascular, nervous system, thymocyte, and palate development. It directs embryonic neuronal axon path finding, and its up regulation on local macrophages following neuronal injury promotes the growth of regenerating axons. EphB3 inhibits colorectal carcinogenesis and invasion by preventing the migration of tumor cells out of the intestinal crypt. Within the ECD, mouse EphB3 shares 96% and 99% amino acid sequence identity with human and rat EphB3, respectively.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using COLO 205 cells.

- Cells may be Fc-blocked with 1 µg of mouse IgG/ $10^5$  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  $1 \times 10^6$  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 Mouse Lyse Buffer (Catalog # FC003).
- 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 PE-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.