Anti-EPHB4 antibodies, human

For research use only

6

One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
Anti-EPHB4-Biotin	for 100 tests	130-115-497
Anti-EPHB4-FITC	for 30 tests	130-115-595
Anti-EPHB4-FITC	for 100 tests	130-115-498
Anti-EPHB4-PE	for 30 tests	130-115-596
Anti-EPHB4-PE	for 100 tests	130-115-499
Anti-EPHB4-APC	for 30 tests	130-115-597
Anti-EPHB4-APC	for 100 tests	130-115-500
Anti-EPHB4-PE-Vio615	for 30 tests	130-115-908
Anti-EPHB4-PE-Vio615	for 100 tests	130-115-836
Anti-EPHB4-PE-Vio770	for 30 tests	130-115-598
Anti-EPHB4-PE-Vio770	for 100 tests	130-115-501
Anti-EPHB4-APC-Vio770	for 30 tests	130-115-599
Anti-EPHB4-APC-Vio770	for 100 tests	130-115-502
Anti-EPHB4-Biotin	for 30 tests	130-115-594

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Technical data and background information

Antigen	EPHB4
Clone	REA923
Isotype	recombinant human IgG1
Isotype control	REA Control (S) antibodies
Alternative names of antigen	Ephrin type-B receptor 4, Hepatoma transmembrane kinase, Tyrosine-protein kinase TYRO11, HTK, MYK1, TYRO11
Entrez Gene ID	2050
Molecular mass of antigen [kDa]	107
Distribution of antigen	placenta, liver, lung, kidney, pancreas, skeletal muscle, breast, colon, endothelial cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA923 recognizes the human ephrin type-B receptor 4 (EPHB4) antigen, which is a member of the Eph receptor tyrosine kinase family. The ligand for EPHB4 is ephrin-B2. EPHB4 is found in different tissues, e.g., in placenta, kidney, liver, lung, pancreas, skeletal muscle, and heart. It is expressed on myeloid hematopoietic cells and endothelial cells. EPHB4 functions as an inhibitor for cell-cell adhesion, chemotaxis, and angiogenesis as well as a promoter for the differentiation of megakaryocytic and erythroid progenitor cells. Additional information: Clone REA923 displays negligible binding to Fc receptors.

Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C).
  Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

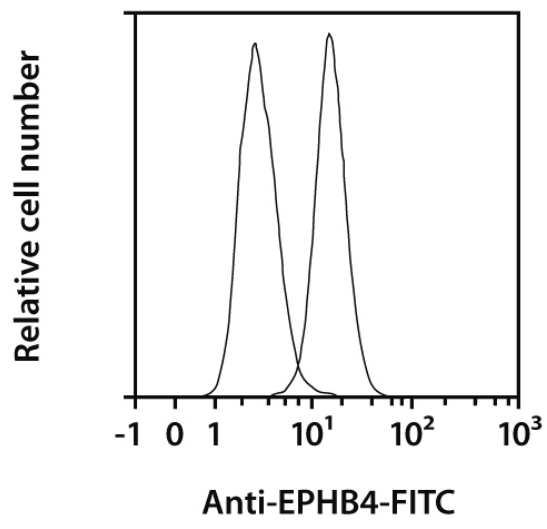
Protocol for cell surface staining

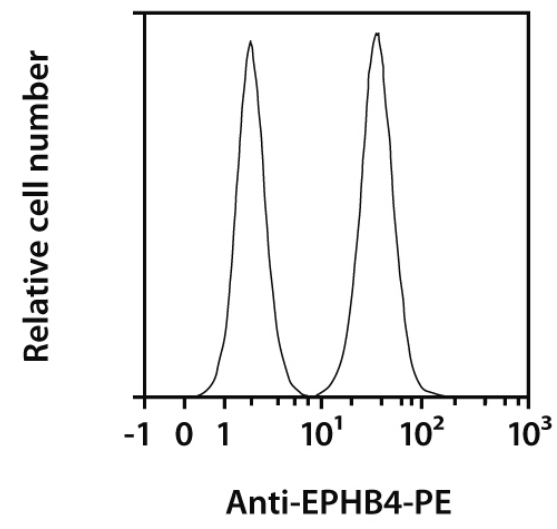
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

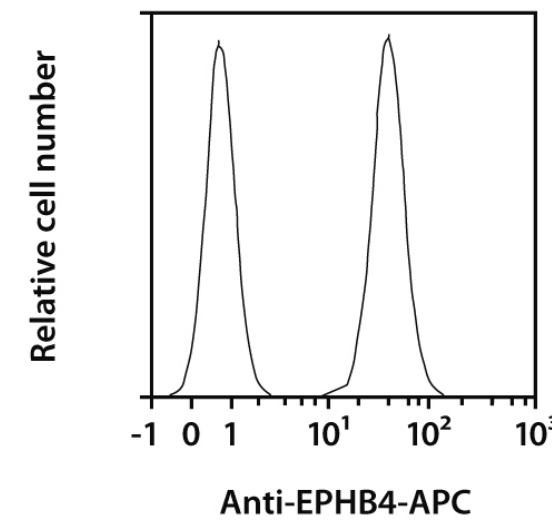
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1−2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

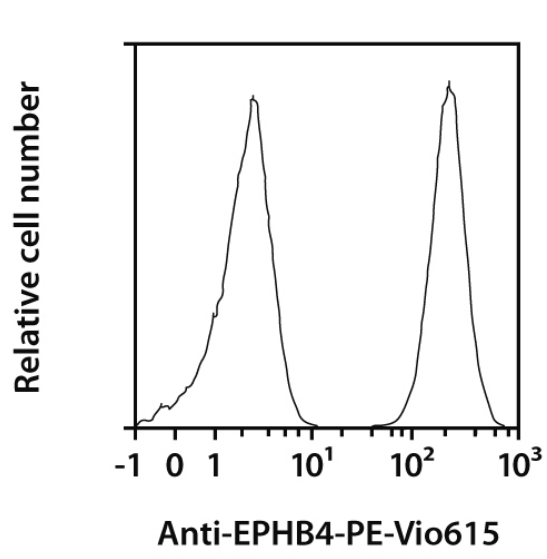
Examples of immunofluorescent staining

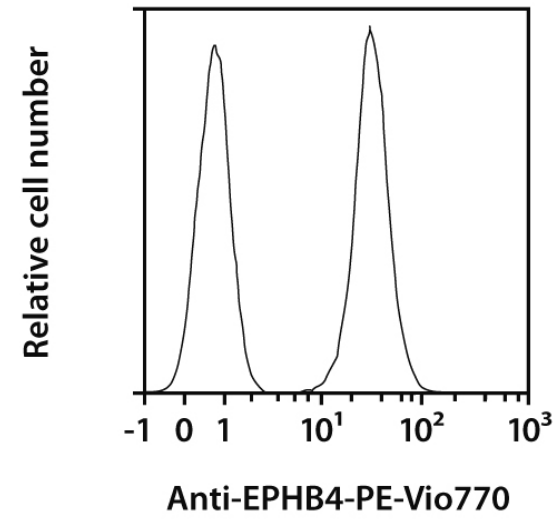
MCF-7 cells were stained with Anti-EPHB4 antibodies or with the corresponding REA Control (S) antibodies (left peak). Flow cytometry was performed using the MACSQuant®Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

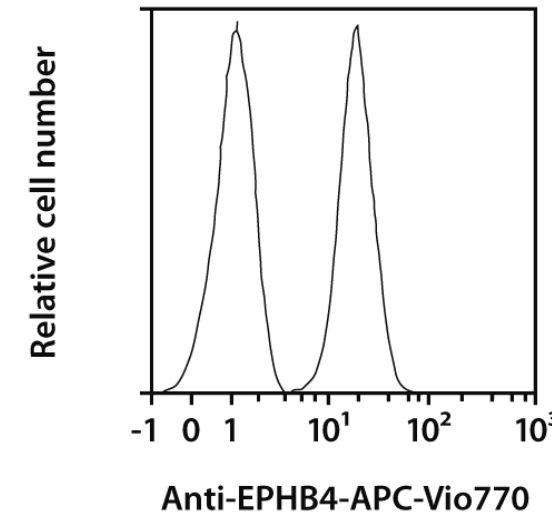


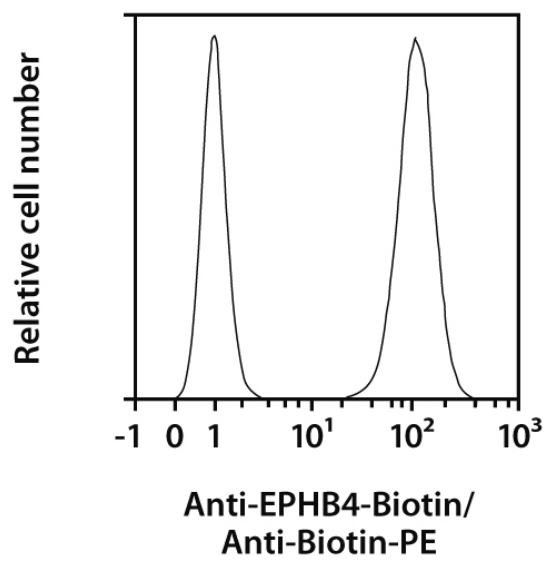












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