# Monoclonal Anti-human ErbB4-Phycoerythrin

**Catalog Number:** FAB11311P
**Lot Number:** AAWL03
**100 Tests**

## Reagents Provided

**Phycoerythrin (PE)-conjugated mouse monoclonal anti-human ErbB4:** Supplied as 25 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 182818

**Isotype:** mouse $IgG_{2A}$

## Reagents Not Provided

- PBS (Dulbecco's PBS)
- BSA

## Storage

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

## Intended Use

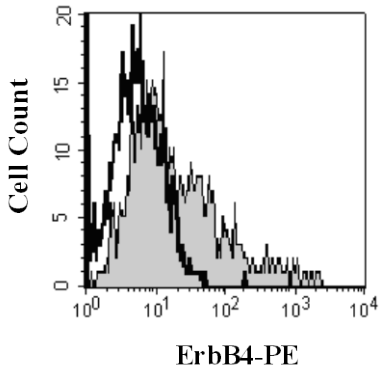
Designed to quantitatively determine the percentage of cells bearing ErbB4 within a population and qualitatively determine the density of ErbB4 on cell surfaces by flow cytometry.

## Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing ErbB4. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing ErbB4 are fluorescently stained, with the intensity of staining directly proportional to the density of expression of ErbB4. Cell surface expression of ErbB4 is determined by flow cytometry using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565 - 605 nm.

## Reagent Preparation

**Phycoerythrin-conjugated mouse anti-human ErbB4:** Use as is; no preparation necessary.




MCF-7 cells were stained with PE-conjugated anti-human ErbB4 (Catalog # FAB11311P, filled histogram) or PE-conjugated isotype control (Catalog # IC003P, open histogram).

## Sample Preparation

**Peripheral blood cells:** Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anticoagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) followed by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells should then be transferred to a 5 mL tube for staining with the monoclonal antibody. Whole blood will require lysis of RBC following the staining procedure.

**Cell Cultures:** Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of $4 \times 10^6$ cells/mL and 25 μL of cells ($1 \times 10^5$) transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from their substrates. Cells that require trypsinization to enable removal from their substrates should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

## Sample Staining

1) Cells should be Fc-blocked by treatment with 1 μg of human IgG/$10^5$ cells for 15 minutes at room temperature prior to staining. Do not wash excess blocking IgG from this reaction.
2) Transfer 25 μL of the Fc-blocked cells (up to $1 \times 10^6$ cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated ErbB4 reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted ErbB4 reagent by washing the cells twice in 4 mL of the same PBS buffer (*Note: Whole blood will require an RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Catalog # WL1000*).
6) Finally, resuspend the cells in 200 - 400 μL of PBS buffer for analysis by flow cytometry.
7) As a control for this analysis, cells in a separate tube should be treated with PE-labeled mouse $IgG_{2A}$ antibody.

This procedure may need modification, depending upon final utilization.



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## Background Information

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant human ErbB4 (rhErbB4), extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. ErbB4 is one of the homologs of the avian erythroblastic leukemia viral oncogene, ErbB. It is a member of the type I receptor tyrosine kinase superfamily that also includes EGF R, ErbB2, and ErbB3. ErbB4 is also known as HER4 because it functions as a receptor for Heregulin.

**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.