Monoclonal Anti-human ErbB2-Allophycocyanin

Catalog Number: FAB1129A
Lot Number: LNN05
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human ErbB2: Supplied as 10 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 191924

Ig Class: mouse IgG_{2B}

Additional Reagents Required

- 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

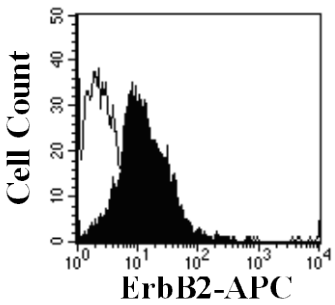
Designed to quantitatively determine the percentage of cells bearing ErbB2 within a population and qualitatively determine the density of ErbB2 on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the APC-labeled monoclonal antibody, which binds to cells expressing ErbB2. Unbound APC-conjugated antibody is then washed from the cells. Cells expressing ErbB2 are fluorescently stained, with the intensity of staining directly proportional to the density of ErbB2. Cell surface expression of ErbB2 is determined by flow cytometric analysis using 620-650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660-670 nm.

Reagent Preparation

APC-conjugated mouse anti-human ErbB2: Use as is; no preparation is necessary.



Human MCF-7 cells stained with APC-conjugated anti-ErbB2 (Catalog # FAB1129A, filled histogram) or APC-conjugated isotype control (Catalog # IC0041A, 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) by centrifugation at 500 x g for 5 minutes. 50 μL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells 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), as described above, 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: *Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate 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 to be used for staining with the antibody may be first Fc-blocked by treatment with 1 μg of mouse or human $IgG/10^5$ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction
2) Transfer 25 μL of the Fc-blocked cells (up to 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of APC-conjugated anti-ErbB2 reagent.
4) Incubate for 30-45 minutes at 2-8 °C.
5) Following this incubation, remove unreacted anti-ErbB2 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Human Erythrocyte Lysing Kit, Catalog # WL1000*).
6) Resuspend the cells in 200-400 μL of PBS buffer for final flow cytometric analysis.
7) As a control for 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 final utilization.

R&D Systems, Inc.
1-800-343-7475

Background Information

The ErbB or Epidermal Growth Factor (EGF) family of receptor tyrosine kinases consists of four members: EGF R/ErbB1/HER1, ErbB2/Neu/HER2, ErbB3/HER3, and ErbB4/HER4. ErbB receptors form homo- and heterodimers leading to the activation of their tyrosine kinase domain and subsequent autocatalytic phosphorylation of specific tyrosine residues in the cytoplasmic tail (1, 2). The ErbB proteins are not only important for the essential roles they play in normal developmental processes, but also for their association with human tumorigenesis (1). ErbB2 is an orphan receptor and heterodimerizes with ErbB1, ErbB3 or ErbB4 to form high affinity receptor complexes for EGF family ligands (3). The neuregulins-driven ErbB2/ErbB3 heterodimer is the most potent and most common ErbB receptor pairing (2, 3). ErbB2 is highly expressed in many cancer types and overexpression of ErbB2 is correlated with a poor prognosis for breast and ovarian cancer patients (1, 4).

References

1. Slamon, D.J. (1989) Science **244**:707.
2. Holbro, T. *et al*. (2003) Exp. Cell Res. **284**:99.
3. Citri, A. *et al*. (2003) Exp. Cell Res. **284**:54.
4. Menard, S. *et al*. (2003) Oncogene **22**:6570.

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.