Monoclonal Anti-human EGF R-Phycoerythrin

Catalog Number: FAB10951P
Lot Number: AAMQ02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-human EGF R: Supplied as 25 μg of antibody in 1 mL PBS containing 0.1% sodium azide.

Clone #: 423103

Isotype: rat 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

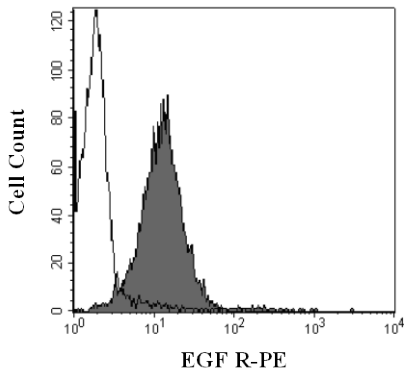
Designed to quantitatively determine the percentage of cells bearing EGF R within a population and qualitatively determine the density of EGF R on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the phycoerythrin-labeled monoclonal antibody, which binds to cells expressing EGF R. Unbound phycoerythrin-conjugated antibody is then washed from the cells. Cells expressing EGF R are fluorescently stained, with the intensity of staining being directly proportional to the density of expression of EGF R. Cell surface expression of EGF R is determined by flow cytometric analysis 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 rat anti-human EGF R: Use as is; no preparation necessary.



A431 cells were stained with PE-conjugated anti-human EGF R (Catalog # FAB10951P, filled histogram) or PE-conjugated isotype control (Catalog # IC006P, 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 4×10^6 cells/mL and 25 μL of cells (1×10^5) 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 1×10^6 cells) or 50 μL of packed whole blood to a 5 mL tube.
3) Add 10 μL of PE-conjugated EGF R reagent.
4) Incubate for 30 - 45 minutes at 2° - 8° C.
5) Following this incubation, remove unreacted EGF R 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 analysis, cells in a separate tube should be treated with PE-labeled rat IgG_{2A} antibody.

This procedure may need modification, depending upon final utilization.

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Background Information

The epidermal growth factor receptor (EGF R) subfamily of receptor tyrosine kinases comprises four members: EGF R (also known as HER1, ErbB1 or ErbB), ErbB2 (Neu, HER-2), ErbB3 (HER-3), and ErbB4 (HER-4). All family members are type I transmembrane glycoproteins that have an extracellular domain containing two cysteine-rich domains separated by a spacer region that is involved in ligand-binding, and a cytoplasmic domain which has a membrane-proximal tyrosine kinase domain, and a C-terminal tail with multiple tyrosine autophosphorylation sites. The human EGF R gene encodes a 1210 amino acid (aa) residue precursor with a 24 aa putative signal peptide, a 621 aa extracellular domain, a 23 aa transmembrane domain, and a 542 aa cytoplasmic domain. EGF R has been shown to bind a subset of the EGF family ligands, including EGF, amphiregulin, TGF-α, betacellulin, epiregulin, heparin-binding EGF, and neuregulin-2α, in the absence of a co-receptor. Ligand binding induces EGF R homodimerization as well as heterdimerization with ErbB2, resulting in kinase activation, tyrosine phosphorylation, and cell signaling. EGF R can also be recruited to form heterodimers with the ligand-activated ErbB3 or ErbB4. EGF R signaling has been shown to regulate multiple biological functions including cell proliferation, differentiation, motility and apoptosis. In addition, EGF R signaling has also been shown to play a role in carcinogenesis.[1 - 3]

References

1. Daly, R.J., 1999, Growth Factors, **16**:255 - 263.
2. Schlessinger, J., 2000, Cell. **103**:211 - 225.
3. Maihle, N.J. *et al*., 2002, Cancer Treat. Res. **107**:247 - 258.

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.