

## Technical Data Sheet

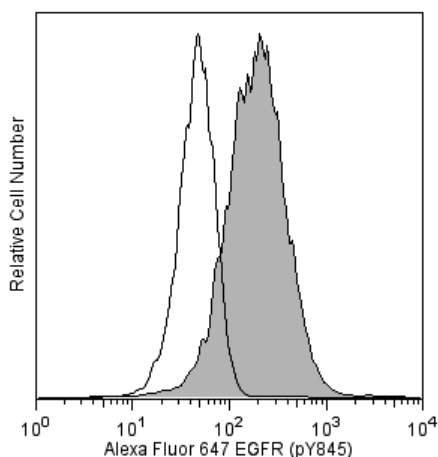
**Alexa Fluor® 647 Mouse anti-EGF Receptor (pY845)****Product Information**

<b>Material Number:</b>	558523
<b>Size:</b>	50 tests
<b>Vol. per Test:</b>	20 µl
<b>Clone:</b>	12A3
<b>Immunogen:</b>	Phosphorylated Human EGF Receptor Peptide
<b>Isotype:</b>	Mouse IgG1, κ
<b>Reactivity:</b>	QC Testing: Human Reported Reactivity: Mouse, Rat
<b>Storage Buffer:</b>	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**

Epidermal Growth Factor (EGF) elicits a variety of cellular responses that are initiated by EGF Receptor (EGFR) binding and activation of intrinsic tyrosine kinase activity. EGFR, also known as ErbB1 or HER1, is a member of the ErbB class of receptor protein tyrosine kinases. It has an extracellular ligand-binding domain, a single transmembrane region, and a cytoplasmic region containing a protein tyrosine kinase domain and a c-terminal regulatory domain with many phosphorylation sites. Following ligand binding, EGFR forms homodimers and heterodimers with ErbB2. Specific C-terminal tyrosine residues are then autophosphorylated and, in turn, bind to adaptor proteins, kinases, or protein tyrosine phosphatases. In addition, c-Src-dependent phosphorylations at other sites, such as tyrosine 845 (Y845) in the kinase domain, regulate the receptor's kinase activity. Inappropriate expression or mutations of EGFR and/or deregulation of its signaling pathways are associated with many types of cancer, making EGFR a promising target for cancer therapies.

The 12A3 monoclonal antibody recognizes the phosphorylated Y845 in the protein tyrosine kinase domain of human EGFR.



**Analysis of EGF Receptor (pY845) in human epidermoid carcinoma.** A-431 cells were either treated for 30 minutes with EGF (Cat. No. 354052 or 356052, shaded histogram) or untreated (open histogram). The cells were fixed (BD Cytofix™ buffer, Cat. No. 554655) for 10 minutes at 37°C, then permeabilized (BD Phosflow™ Perm Buffer III, Cat. No. 558050) on ice for 30 minutes, and then stained with Alexa Fluor® 647 Mouse anti-EGF Receptor (pY845). Flow cytometry was performed on a BD FACSAArray™ bioanalyzer system.

**Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

**Application Notes****Application**

Intracellular staining (flow cytometry)	Routinely Tested
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**Suggested Companion Products**

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

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## Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^6$  cells in a 100- $\mu$ l experimental sample (a test).
2. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
7. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at [www.bdbiosciences.com/colors](http://www.bdbiosciences.com/colors).
8. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

## References

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