## **Technical Data Sheet**

# Purified Mouse anti-EGF Receptor (pY845)

#### **Product Information**

 Material Number:
 558381

 Size:
 0.1 mg

 Concentration:
 0.5 mg/ml

 Clone:
 12A3

Immunogen: Phosphorylated Human EGF Receptor Peptide

Reported Reactivity: Mouse, Rat

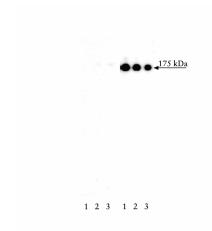
Target MW: 175 kDa

Storage Buffer: Aqueous buffered solution containing ≤0.09% sodium azide.

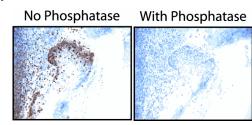
### Description

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.



Western blot analysis of EGF Receptor (pY845) in human epidermis. Lysates from control (left panel) and EGF-treated (Cat. No. 354052, right panel) human A-431 epidermoid carcinoma were probed with purified mouse anti-EGF Receptor (pY845) monoclonal antibody at concentrations of 0.1, 0.05, and 0.025 µg/ml (lanes 1, 2, and 3, respectively). EGF Receptor (pY845) is identified as a band of 175 kDa in the treated cells.



EGF Receptor (pY845) staining on tonsil. Fresh human tonsil was incubated in 5 mM Pervanadate solution for 2 hours, then fixed in formalin and processed. Following antigen retrieval with BD Retrievagen A buffer (Cat. no. 550524), the sections were either left untreated (left panel) or treated with a phosphatase to eliminate all phosphorylation (right panel). The tissue sections were stained with purified Mouse anti-EGF Receptor (pY845) with Hematoxylin counterstaining. No staining was seen on sections of unstimulated tonsil (data not shown). Original magnification: 20X.

### **Preparation and Storage**

Store undiluted at 4°C.

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

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#### **Application Notes**

#### Application

| -FF  |                  |
|--|------------------|
| Western blot   | Routinely Tested |
| Immunohistochemistry-formalin (antigen retrieval required) | Reported         |
| ELISA  | Reported         |

#### **Suggested Companion Products**

| Catalog Number | Name                   | Size   | Clone  |
|----------------|------------------------|--------|--------|
| 554002         | HRP Goat Anti-Mouse Ig | 1.0 ml | (none) |

## **Product Notices**

- 1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
- 2. 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.
- 3. Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE (No Azide/Low Endotoxin) antibody format, if available, for in vitro and in vivo use.
- Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

#### References

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Boerner JL, Demory ML, Silva C, Parsons SJ. Phosphorylation of Y845 on the epidermal growth factor receptor mediates binding to the mitochondrial protein cytochrome c oxidase subunit II. Mol Cell Biol. 2004; 24(16):7059-7071. (Biology)

Hristozova T, Konschak R, Budach V, Tinhofer I. A simple multicolor flow cytometry protocol for detection and molecular characterization of circulating tumor cells in epithelial cancers. Cytometry A. 2012; 81A(6):489-495. (Clone-specific: Flow cytometry)

Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. *J Clin Oncol.* 2003; 21(14):2787-2799. (Biology)

Moro L, Dolce L, Cabodi S, et al. Integrin-induced epidermal growth factor (EGF) receptor activation requires c-Src and p13Cas and leads to phosphorylation of specific EGF receptor tyrosines. *J Biol Chem.* 2002; 277(11):9405-9414. (Biology)

Olayioye MA, Neve RM, Lane HA, Nynes NE. The ErbB signaling network: receptor heterodimerization in development and cancer. *EMBO J.* 2000; 19(13):3159-3167. (Biology)

Wu W, Graves LM, Gill GN, Parsons SJ, Samet JM. Src-dependent phosphorylation of the epidermal growth factor receptor on tyrosine 845 is required for zinc-induced Ras activation. *J Biol Chem.* 2002; 277(27):24252-24257. (Biology)

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