Human EGFR Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF231

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human EGFR in ELISAs and Western blots. In sandwich ELISAs, approximately 3% cross-reactivity with recombinant mouse EGFR is observed and less than 0.1% cross-reactivity with recombinant human (rh) ErbB2 and rhErbB3 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human EGFR Leu25-Ser645 Accession # CAA25240
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

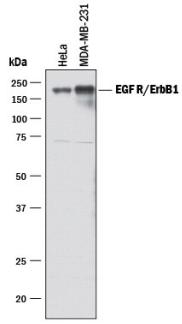
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	See Below
Immunocytochemistry	1-15 µg/mL	See Below
Immunohistochemistry	1-15 µg/mL	See Below
Immunoprecipitation	1 µg/mL	A431 human epithelial carcinoma cell line, see our available Western blot detection antibodies
Human EGFR Sandwich Immunoassay		**Reagent**
ELISA Capture	0.2-0.8 µg/mL	Human EGFR Antibody (Catalog # AF231)
ELISA Detection	0.1-0.4 µg/mL	Human EGFR Biotinylated Antibody (Catalog # BAF231)
Standard		Recombinant Human EGFR (Catalog # 1095-ER)
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

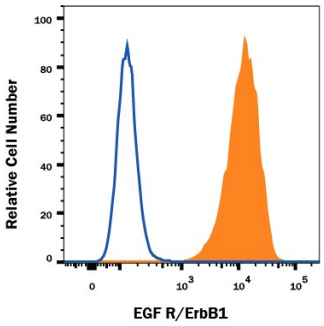
DATA

Western Blot



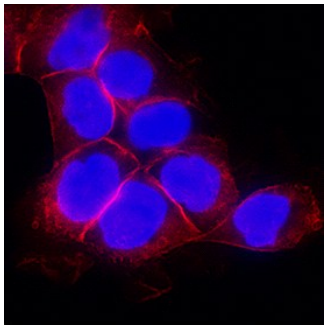
Detection of Human EGFR by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and MDA-MB-231 human breast cancer cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human EGFR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF231) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for EGFR at approximately 175 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



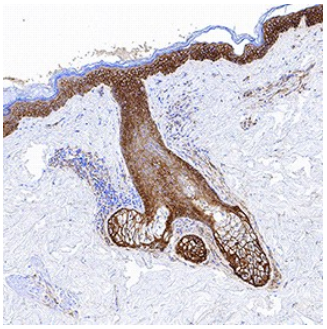
Detection of EGFR in A431 Human Cell Line by Flow Cytometry. A431 human epithelial carcinoma cell line was stained with Goat Anti-Human EGFR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF231, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Phycoerythrin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0107). View our protocol for Staining Membrane-associated Proteins.

Immunocytochemistry



EGFR in A431 Human Cell Line. EGFR was detected in immersion fixed A431 human epithelial carcinoma cell line using Goat Anti-Human EGFR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF231) at 1 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to plasma membrane. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



EGFR in Human Skin. EGFR was detected in immersion fixed frozen sections of human skin using Goat Anti-Human EGFR Antigen Affinity-purified Polyclonal Antibody (Catalog # AF231) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membrane. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

The epidermal growth factor receptor (EGFR) subfamily of receptor tyrosine kinases comprises four members: EGFR (also known as HER1, ErbB1 or ErbB), ErbB2 (Neu, HER2), ErbB3 (HER3), and ErbB4 (HER4). All family members are type I transmembrane glycoproteins that have an extracellular domain which contains 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 EGFR 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. EGFR 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 EGFR homodimerization as well as heterodimerization with ErbB2, resulting in kinase activation, tyrosine phosphorylation and cell signaling. EGFR can also be recruited to form heterodimers with the ligand-activated ErbB3 or ErbB4. EGFR signaling has been shown to regulate multiple biological functions including cell proliferation, differentiation, motility and apoptosis. In addition, EGFR signaling has also been shown to play a role in carcinogenesis (1 - 3).

References:

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

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