Human Amphiregulin Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF262

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Amphiregulin in direct ELISAs and Western blots. In direct ELISAs, less than 5% cross-reactivity with recombinant mouse (rm) Amphiregulin, rmEpiregulin, recombinant human (rh) HB-EGF, and rhTGF-α is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Amphiregulin Val107-Lys184 Accession # P15514
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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 either lyophilized or as a 0.2 µm filtered solution in PBS.

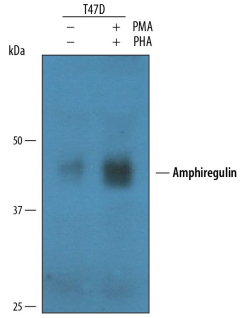
APPLICATIONS

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
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Neutralization	Measured by its ability to neutralize Amphiregulin-induced proliferation in the Balb/3T3 mouse embryonic fibroblast cell line. The Neutralization Dose (ND_{50}) is typically 0.3-1 µg/mL in the presence of 50 ng/mL Recombinant Human Amphiregulin.	

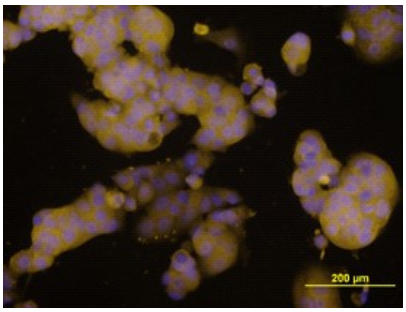
DATA

Western Blot



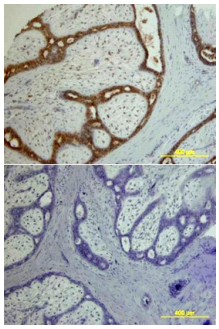
Detection of Human Amphiregulin by Western Blot. Western blot shows conditioned media from T47D human breast cancer cell line untreated (-) or treated (+) with PMA and PHA for 3 days. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF262) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for Amphiregulin at approximately 45 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



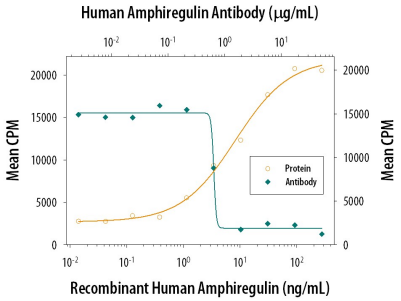
Amphiregulin in MCF-7 Human Cell Line. Amphiregulin was detected in immersion fixed MCF-7 human breast cancer cell line using Goat Anti-Human Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF262) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (yellow; Catalog # NL001) and counter-stained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Amphiregulin in Human Breast. Amphiregulin was detected in immersion fixed paraffin-embedded sections of human breast using Goat Anti-Human Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF262) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Neutralization



Cell Proliferation Induced by Amphiregulin and Neutralization by Human Amphiregulin Antibody. Recombinant Human Amphiregulin (Catalog # 262-AR) stimulates proliferation in the Balb/3T3 mouse embryonic fibroblast cell line in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Human Amphiregulin (50 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF262). The ND_{50} is typically 0.3-1 µg/mL.

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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

Amphiregulin (AR) is a member of the EGF family of cytokines which is comprised of at least ten proteins including EGF, TGF-α, HB-EGF, and the various heregulins. All of these cytokines are synthesized as transmembrane precursors and are characterized by the presence of one or several EGF structural units in their extracellular domain. The soluble forms of these cytokines are released by proteolytic cleavage. Amphiregulin was originally isolated from the conditioned media of a PMA-treated MCF-7 human breast carcinoma cell line. The AR cDNA encodes a 252 amino acid (aa) residue transmembrane precursor. Multiple forms of native AR containing either 78 or 84 aa residues and both N- and O-linked oligosaccharides have been found. Amphiregulin mRNA expression can be detected in numerous carcinoma cell lines and the epithelial cells of various human tissues including colon, stomach, breast, ovary, kidney, etc.

Human AR stimulates the proliferation of various human and mouse keratinocytes, mammary epithelial cells and some fibroblasts. AR is also a growth inhibitor for various tumor cell lines. In certain colon carcinoma cell lines, AR has been shown to be an autocrine growth factor. Amphiregulin can bind to the EGF receptor. It has been suggested that in certain cell types, AR bioactivity may be mediated through the EGF receptor. The 98 aa residue long form of recombinant amphiregulin has shown to be approximately 5 - 10 fold more active than the 78 aa residue form of recombinant AR in an *in vitro* proliferation assay using Balb/3T3 fibroblasts.

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