

DESCRIPTION

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|---------------------------|---|
| Species Reactivity | Mouse |
| Specificity | Detects mouse Amphiregulin in ELISAs and Western Blots. In sandwich immunoassays, less than 1% cross-reactivity with recombinant human Amphiregulin is observed. In Western blots, no cross reactivity with recombinant mouse HB-EGF is observed. |
| Source | Polyclonal Goat IgG |
| Purification | Antigen Affinity-purified |
| Immunogen | <i>E. coli</i> -derived recombinant mouse Amphiregulin Ser94-Lys191 Accession # P31955 |
| 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 as a 0.2 µm filtered solution in PBS. |

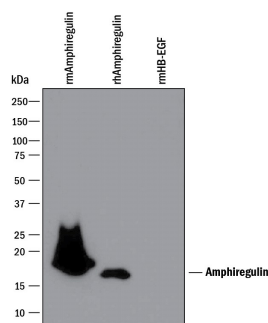
APPLICATIONS

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 | 0.1 µg/mL | See Below |
| Immunohistochemistry | 5-15 µg/mL | See Below |
| Mouse Amphiregulin Sandwich Immunoassay | | Reagent |
| ELISA Capture | 0.2-0.8 µg/mL | Mouse Amphiregulin Antibody (Catalog # AF989) |
| ELISA Detection | 0.1-0.4 µg/mL | Mouse Amphiregulin Biotinylated Antibody (Catalog # BAF989) |
| Standard | | Recombinant Mouse Amphiregulin (Catalog # 989-AR) |
| 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 0.3-1.5 µg/mL in the presence of 50 ng/mL Recombinant Mouse Amphiregulin. | |

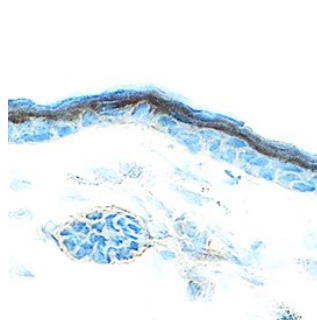
DATA

Western Blot



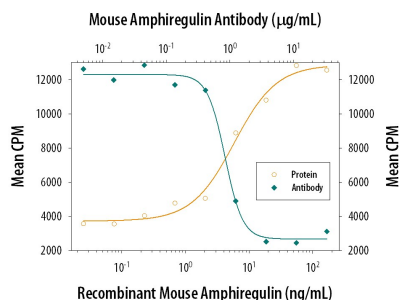
Detection of Recombinant Human and Mouse Amphiregulin by Western Blot. Western blot shows 25 ng of Recombinant Mouse Amphiregulin (Catalog # 989-AR), Recombinant Human Amphiregulin (Catalog # 262-AR), and Recombinant Mouse HB-EGF. PVDF Membrane was probed with 0.1 µg/mL of Goat Anti-Mouse Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF989) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for Amphiregulin at approximately 16-25 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 3.

Immunohistochemistry



Amphiregulin in Mouse Skin. Amphiregulin was detected in immersion fixed frozen sections of mouse skin using Goat Anti-Mouse Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF989) 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). Specific staining was localized to keratinocytes. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Neutralization



Cell Proliferation Induced by Amphiregulin and Neutralization by Mouse Amphiregulin Antibody. Recombinant Mouse Amphiregulin (Catalog # 989-AR) stimulates proliferation in the Balb/3T3 mouse embryonic fibroblast cell line in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Mouse Amphiregulin (50 ng/mL) is neutralized (green line) by increasing concentrations of Mouse Amphiregulin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF989). The ND₅₀ is typically 0.3-1.5 µg/mL.

PREPARATION AND STORAGE

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|--------------------------------|--|
| 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. <ul style="list-style-type: none"> ● 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), also known as Schwannoma-derived growth factor (SDGF), is a member of the epidermal growth factor (EGF) family of growth factors which includes, AR, EGF, transforming growth factor- α (TGF- α), heparin binding EGF-like growth factor (HB-EGF), betacellulin (BTC), epiregulin, and the neuregulins-1 through -4. All EGF family members are synthesized as type I transmembrane precursors and contain one or several EGF domains in their extracellular region. The bioactive form of the proteins is released by proteolytic cleavage. The ErbB family of receptors that includes ErbB1-B4, mediates the biological activities of the EGF family ligands. AR was originally isolated from the conditioned media of PMA-treated MCF-7 human breast carcinoma cell line. AR mRNA expression can be detected in numerous carcinoma cell lines and in the epithelial cells of various human tissues including colon, stomach, breast, ovary and kidney. AR stimulates the proliferation of keratinocytes, mammary epithelial cells, fibroblasts, astrocytes and glial cells. AR is also a growth inhibitor for certain tumor cells. The gene for AR has been mapped to human chromosome 4q13-q21 and mouse chromosome 5. Human and mouse AR cDNA encode 252 and 248 amino acid residue type I membrane proteins, respectively. The two proteins share approximately 69% sequence identity. Mouse AR also shares 81% amino acid sequence homology with rat AR. Several secreted isoforms of AR that vary in length and/or glycosylation level can be found in cell conditioned media. The 98 amino acid residue recombinant AR has better receptor binding and biological activity than the C-terminal truncated forms of the protein.

References:

1. Thompson, S.A. *et al.* (1996) *J. Biol. Chem.* **271**:17927.
2. Sonoda, H. *et al.* (1992) *Biochem. Biophys. Res. Commun.* **185**:103.
3. Normanno, N. *et al.* (2001) *Frontiers in Bioscience* **6**:685.