

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human HB-EGF in ELISAs and Western blots. In direct ELISAs, less than 1% cross reactivity with recombinant mouse HB-EGF is observed. In sandwich immunoassays, less than 0.1% cross-reactivity with recombinant human (rh) Amphiregulin, rhBetacellulin, rhEpregeulin, and recombinant mouse Epigen is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>S. frugiperda</i> insect ovarian cell line Sf 21-derived recombinant human HB-EGF (R&D Systems, Catalog # 259-HE) Asp63-Leu148 Accession # Q99075
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.

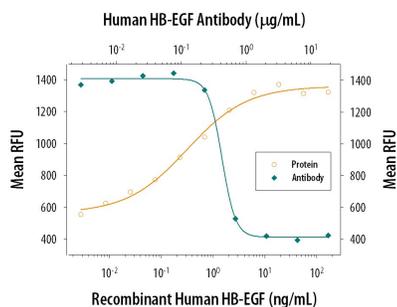
APPLICATIONS

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	0.1 µg/mL	Recombinant Human HB-EGF (Catalog # 259-HE)
Immunohistochemistry	5-15 µg/mL	See Below
Human HB-EGF Sandwich Immunoassay		Reagent
ELISA Capture	0.2-0.8 µg/mL	Human HB-EGF Antibody (Catalog # AF-259-NA)
ELISA Detection	0.1-0.4 µg/mL	Human HB-EGF Biotinylated Antibody (Catalog # BAF259)
Standard		Recombinant Human HB-EGF (Catalog # 259-HE)
Neutralization		Measured by its ability to neutralize HB-EGF-induced proliferation in the Balb/3T3 mouse embryonic fibroblast cell line. Rubin, J.S. <i>et al.</i> (1991) PNAS 88 :415. The Neutralization Dose (ND ₅₀) is typically 0.1-0.6 µg/mL in the presence of 20 ng/mL Recombinant Human HB-EGF.

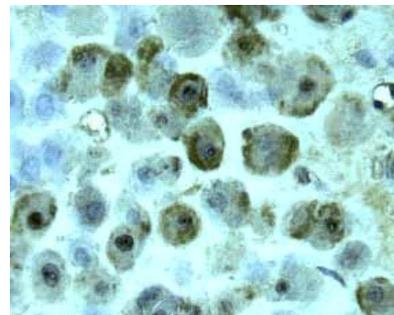
DATA

Neutralization



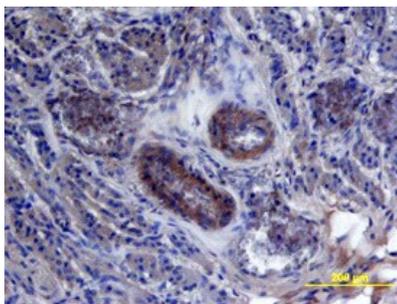
Cell Proliferation Induced by HB-EGF and Neutralization by Human HB-EGF Antibody. Recombinant Human HB-EGF (Catalog # 259-HE) stimulates proliferation in the Balb/3T3 mouse embryonic fibroblast cell line in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Human HB-EGF (20 ng/mL) is neutralized (green line) by increasing concentrations of Human HB-EGF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-259-NA). The ND₅₀ is typically 0.1-0.6 µg/mL.

Immunohistochemistry



HB-EGF in Human Placenta. HB-EGF was detected in immersion fixed paraffin-embedded sections of human placenta using 15 µg/mL Human HB-EGF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-259-NA) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Immunohistochemistry



HB-EGF in Human Placenta. HB-EGF was detected in immersion fixed paraffin-embedded sections of human placenta using Human HB-EGF Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-259-NA) 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). View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <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

HB-EGF was originally purified based on its heparin-binding property and mitogenic activity on BALB-3T3 fibroblasts from the conditioned medium of the human U-937 histiocytic lymphoma cell line. The natural protein has an apparent molecular mass of 19-23 kDa and exists in multiple forms as a result of heterogenous O-glycosylation and/or N-terminal truncation. In addition to fibroblasts, HB-EGF is also a potent mitogen for keratinocytes and smooth muscle cells but not for capillary endothelial cells. HB-EGF is produced in monocytes and macrophages. In addition, transcription of HB-EGF can be induced in vascular endothelial cells as well as aortic smooth muscle cells (SMC), suggesting that HB-EGF may have an important role in the pathogenesis of atherosclerosis.

HB-EGF is a member of the EGF family of mitogens which also include transforming growth factor- α (TGF- α), amphiregulin (AR), rat schwannoma-derived growth factor (SDGF), vaccinia growth factor (VGF), and the various ligands for the Her2/ErbB2/neu receptor. All these cytokines are derived from transmembrane precursors that contain one or several EGF structural units in their extracellular domain. Many of these transmembrane precursors are biologically active and seem to play a role in juxtacrine stimulation of adjacent cells. The cDNA for HB-EGF encodes a 204 amino acid residue transmembrane protein that is proteolytically cleaved to generate the soluble HB-EGF. Like EGF, TGF- α , and AR; HB-EGF binds to the EGF receptor and activates the receptor tyrosine kinase. HB-EGF is reported to be a more potent SMC mitogen than EGF. It has been suggested that the differential activities found for HB-EGF compared to EGF may be mediated by the heparin-binding properties of HB-EGF. A diphtheria toxin receptor that mediates the endocytosis of the bound toxin has been cloned and found to be identical to the transmembrane HB-EGF precursor.