SYMANSIS
CELL SIGNALING SCIENCE

Product Data Sheet

human cell expressed Amphiregulin (mature form)A

Source	A DNA sequence encoding the human Amphiregulin protein sequence (containing the signal peptide, N- and C-terminal propeptide and the mature Amphiregulin sequence) was expressed in modified human 293 cells. Proteolytic processing of the expressed membrane-bound precursor protein generates mature soluble Amphiregulin.
Molecular Mass	Under reducing conditions Symansis Amphiregulin (mature form)B migrates as a broad band between 17.6 and 23.4 kDa on SDS-PAGE due to post-translational modifications, in particular glycosylation. This compares with unmodified Amphiregulin polypeptide that has a predicted monomeric molecular mass of 9.07 kDa.
% Carbohydrate	Symansis purified Amphiregulin (mature form)B consists of 25–50% carbohydrate by weight.
Glycosylation	Symansis Amphiregulin (mature form)B contains N- and O-linked oligosaccharides.
Purity	>95%, as determined by SDS-PAGE, visualized by Coomassie Brilliant Blue.
Formulation	When reconstituted in 0.5 ml sterile 5mM acetic acid, the solution will contain 1% human serum albumin (HSA) and 10% trehalose.
Reconstitution	It is recommended that 0.5 ml of sterile 5mM acetic acid be added to the vial.
Storage	Lyophilized products should be stored at 2 to 8°C. Following reconstitution short-term storage at 4°C is recommended, with longer-term storage in aliquots at -18 to -20°C. Repeated freeze thawing is not recommended.
Activity	The ED_{50} of Amphiregulin (mature form)B is typically 10–15 ng/ml as measured in a cell proliferation assay using the murine Balb/3T3 fibroblast cell line.
Background Information	Amphiregulin is an epidermal growth factor (EGF)-related glycoprotein that is expressed in a variety of tissues including ovary, placenta, lung, kidney, stomach, colon and breast. As an EGF-related growth factor, Amphiregulin is involved in the differentiation and proliferation of a wide range of cell types and these actions are mediated through binding to the EGF receptor. Amphiregulin plays an important role in neoplastic disease states. It has been demonstrated that Amphiregulin is over-expressed in a wide spectrum of cancers including prostate, colorectal, mammary, kidney, bladder, ovary, pancreas, lung, and gliomas. Amphiregulin levels also correlate with reduced survival in patients with non-small cell lung cancer and pancreatic cancer. Therefore, Amphiregulin may promote tumor progression in an autocrine fashion. Conversely, studies have also shown that Amphiregulin can inhibit proliferation of certain cancer cell lines such as A431 human epidermoid cancer cells. Recent studies indicate that Amphiregulin may also act as a protective factor in response to liver damage. Amphiregulin is synthesized as a 252 amino acid precursor glycoprotein with 3 potential N-glycosylation sites. It comprises a 19 amino acid signal peptide and 2 propeptide domains at the N-terminus and C-terminus. The C-terminus propeptide acts as a potential transmembrane domain. The mature form of amphiregulin is reported to be 84aa in length, however the *in vitro* TACE cleavage site has been shown to be 3 aa downstream generating a mature protein of 87aa. Furthermore, the recombinant 87aa form has been shown to be more active than the recombinant 84aa form. Symansis Amphiregulin (mature form)B h as been confirmed to be 87aa. For a recent paper documenting a novel role for Amphiregulin please refer to Berasain C *et al.* (2005) *J Biol Chem* **280**(19): 19012-20.

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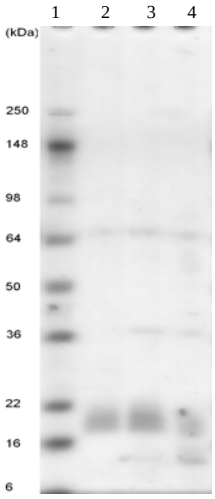
1D gel



1D gel data	Lane 1– MW markers; Lane 2– Amphiregulin (mature form)B ; Lane 3– Amphiregulin (mature form)B treated with PNGase F to remove potential N-linked glycans; Lane 4–Amphiregulin (mature form)B treated with a glycosidase cocktail to remove potential N- and O-linked glycans. Approximately 5 µg of protein was loaded per lane; Gel was stained using Coomassie. Drop in MW after treatment with PNGase F indicates presence of N-linked glycans. A further drop in MW after treatment with the glycosidase cocktail indicates the presence of O-linked glycans.
Theoretical Sequence	VVKPPQNKTESENTSDKPKRKKKGGKNGKNRRNRKKKNPCNAEFQNFCIHGECKYIEH LEAVTCKCQQEYFGERCGEKSMK N-terminal and C-terminal sequences confirmed by Edman Sequencing and LC-MS, respectively.

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