



Recombinant Human Sonic Hedgehog N-Terminus, C24II Substitution

Catalog Number: 1845-SH/CF

Background

Human Shh cDNA encodes a 462 amino acid (aa) residue (45kDa) precursor protein with a 23 aa signal peptide. An autocatalytic cleavage reaction yields a 19 kDa (residues 24 - 197) amino-terminal fragment (Shh-N), and a 25 kDa (residues 198 - 462) carboxy-terminal domain (Shh-C). The N-terminal domain retains all known signaling capabilities, while the C-terminal domain is responsible for the intramolecular processing, acting as a cholesterol transferase that covalently transfers the cholesterol molecule to the C-terminus of Shh-N. When Shh is expressed in insect or mammalian cells, a palmitoyl group is also attached to the N-terminal cysteine of Shh-N via an amide linkage. Although the binding affinity to their receptors is not changed, lipid-modified Shh-N proteins are more potent than the unmodified proteins in cell-based assays. Other hydrophobic modifications to unmodified Shh-N, including the substitution of the N-terminal cysteine residue with two hydrophobic isoleucine residues, can also increase Shh-N potency. At the cell surface, Shh-N activity is mediated by a multicomponent receptor complex involving the 12-pass transmembrane protein, Patched (Ptc) which binds Shh with high affinity and Smoothed (Smo), a signaling seven transmembrane G-protein coupled receptor. In the absence of Shh, Ptc represses Smo activity. The binding of Shh to Ptc releases the basal repression of Smo by Ptc. Shh is expressed in key embryonic tissues such as the Hensen's node, the zone of polarizing activity in the posterior limb bud, the notochord, and the floor plate of the neural tube. Shh is involved in regulating the patterning of the developing central nervous system, somite, and limb. Shh plays an important role in the development of particular tissues such as whisker, hair, foregut, tooth and bone. Some evidence also suggests that Shh is involved in regulating stem cell fates of neural and hematopoietic lineages and that aberrant Shh signaling is implicated in basal cell carcinomas and other diseases (1 - 5).

References:

1. Carpenter, D. *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**:13630.
2. Ingham, P. and A. McMahon (2001) *Genes & Dev.* **15**:3059.
3. Mullor, J. *et al.* (2002) *Trends Cell Biol.* **12**:562.
4. Perrimon, N. (1995) *Cell* **80**:517.
5. Taylor, F. *et al.* (2001) *Biochemistry* **40**:4359.

Description

Source	<i>E. coli</i> -derived Cys24 - Gly197 (Cys24Ile-Ile), with an N-terminal Met Accession # NP_000184
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	20 kDa

Specifications

Activity	Measured by its ability to induce alkaline phosphatase production by C3H10T1/2 mouse fibroblasts. Nakamura, T. <i>et al.</i> (1997) <i>Biochem. Biophys. Res. Commun.</i> 237 :465. The ED ₅₀ for this effect is typically 0.1 - 0.4 µg/mL.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS and NaCl. See Certificate of Analysis for details.

Preparation and Storage

Reconstitution	Reconstitute at 100 µg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
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.● 3 months, -20 to -70 °C under sterile conditions after reconstitution.

7/31/2009

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