

DESCRIPTION

Source *E. coli*-derived
Cys28-Gly202 (Cys28Ile-Ile), with an N-terminal Met
Accession # P97812.2

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 embryonic fibroblast cells. Nakamura, T. *et al.* (1997) *Biochem. Biophys. Res. Commun.* **237**:465.
The ED₅₀ for this effect is typically 3-12 µg/mL.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >97%, by SDS-PAGE under reducing conditions and visualized by silver stain.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS and NaCl with Trehalose. 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.

- 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.

BACKGROUND

The *hedgehog* (*hh*) gene encoding a secreted protein was originally identified in *Drosophila* as a segment polarity gene. The vertebrate homologues of Hh comprise several proteins including sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) (1). Hedgehog proteins are important signaling molecules during embryonic development and are highly conserved within and across species (1). Mouse and human Ihh share 100% amino acid identity in the signaling domain, while mouse Ihh and Shh share 90% amino acid identity in the N-terminal signaling domain. Ihh mRNA expression is detected in fetal lung, gut, stomach, liver, kidney, pancreas and strongly in cartilage - in growth regions of the developing bone (2, 3). Ihh, along with parathyroid hormone related protein, regulate the rate of chondrocyte proliferation and differentiation (4). Ihh is also involved in yolk sac vasculogenesis, playing an important role in differentiation of epiblast cells into endothelial and red blood cells (5).

Mouse Ihh cDNA encodes a 411 amino acid (aa) polypeptide with a predicted 27 aa signal peptide. This polypeptide is cleaved to generate a 45 kDa precursor protein that undergoes the same post-translation processing as Shh (3). An autocatalytic reaction yields a 19 kDa amino-terminal domain Ihh-N protein that retains all known signaling capabilities, and a 23 kDa carboxy-terminal domain Ihh-C protein (3). Since hydrophobic modifications to Shh, including the substitution of the N-terminal cysteine residue with two hydrophobic isoleucine residues, can also increase its potency (6), a similar modification was made for Ihh. This modified form also shows increased potency in a bioassay measuring induction of alkaline phosphatase. At the cell surface, Hedgehog activity is mediated by a multicomponent receptor complex involving the 12-pass transmembrane protein Patched (Ptc) which binds Hedgehogs with high affinity and Smoothened (Smo), a signaling seven transmembrane G-protein coupled receptor (1).

References:

1. Ingham, P. and A. McMahon (2001) *Genes & Dev.* **15**:3059.
2. Marigo, V. *et al.* (1995) *Genomics* **28**:44.
3. Valentini, R.P. *et al.* (1997) *J Biol Chem.* **272**:8466.
4. Vortkamp, A. *et al.* (1996) *Science* **273**:613.
5. Byrd, N. *et al.* (2002) *Development* **129**:361.
6. Taylor, F.R. *et al.* (2001) *Biochemistry* **40**: 4359.