

DESCRIPTION

Source *E. coli*-derived human Sonic Hedgehog/Shh protein
Cys24-Gly197 (Cys24Ile-Ile), with an N-terminal Met
Accession # NP_000184
Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under current Good Manufacturing Practice (GMP) guidelines.

N-terminal Sequence Analysis Met-Ile-Ile-Gly₂₅-Pro-Gly-Arg-Gly-Phe-Gly

Predicted Molecular Mass 20 kDa

SPECIFICATIONS

SDS-PAGE 22 kDa, reducing conditions

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 0.1-0.4 µg/mL.

Endotoxin Level <0.01 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE with silver staining, under reducing conditions.

Host Cell Protein < 0.5 ng per µg of protein when tested by ELISA.

Mycoplasma Negative when tested in a ribosomal RNA hybridization assay.

Formulation Lyophilized from a 0.2 µm filtered solution in PBS and NaCl. See Certificate of Analysis for details.

PREPARATION AND STORAGE

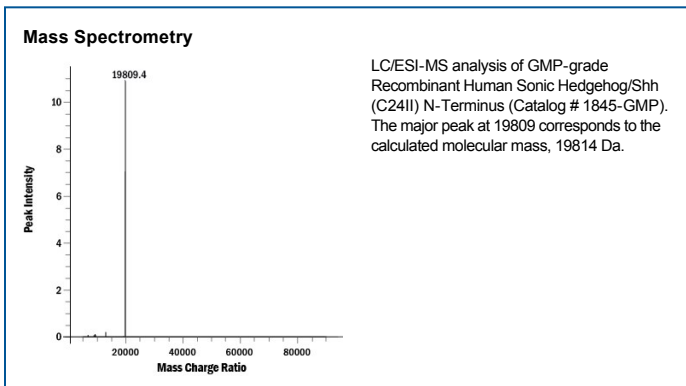
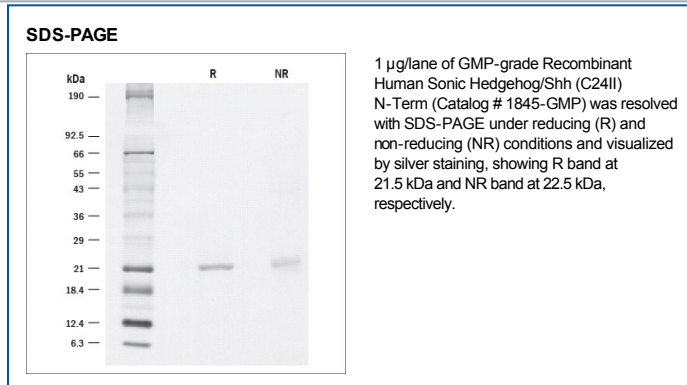
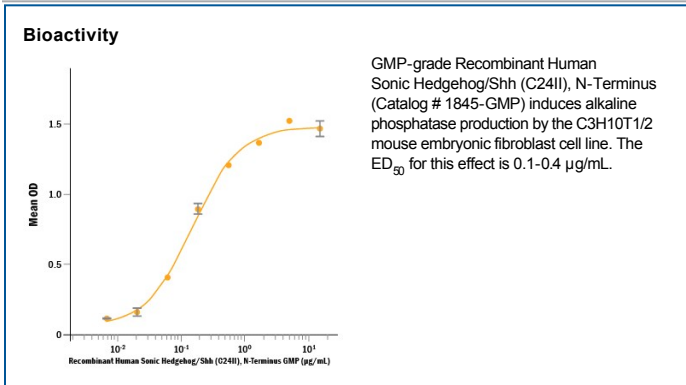
Reconstitution Reconstitute at 100-200 µg/mL in PBS, and allow up to 24 hours at 2 to 8 °C for complete reconstitution.

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.**

- A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 3 months, ≤ -20 °C under sterile conditions after reconstitution.

DATA



BACKGROUND

Sonic Hedgehog (Shh) is expressed in embryonic tissues that are critical for the patterning of the developing central nervous system, somite, and limb. It is also involved in whisker, hair, foregut, tooth, and bone development. Shh regulates neural and hematopoietic stem cell fate and is important for thymocyte differentiation and proliferation as well as T cell determination. In adult tissue Shh is associated with cancer development and tissue remodeling following injury (1-3). Human Shh encodes a 462 amino acid (aa) precursor protein that is autocatalytically processed to yield a non-glycosylated 19 kDa N-terminal fragment (Shh-N) and a glycosylated 25 kDa C-terminal protein (Shh-C) (4). Shh-C, which is responsible for the intramolecular processing of Shh, is rapidly degraded following Shh proteolysis (5). Shh-N is highly conserved, sharing >98% aa identity between mouse, human, rat, canine, porcine, and chicken Shh-N. Shh-N can be palmitoylated at its N-terminal cysteine and modified by cholesterol addition at its C-terminus (6). These modifications contribute to the membrane tethering of Shh as well as its assembly into various sized multimers (6-9). Lipid modification and multimerization greatly increase Shh-N receptor binding affinity and signaling potency (5, 6, 8, 9). Monomeric and multimeric Shh can be released from the plasma membrane by the cooperative action of DISP1, SCUBE2, and TACE/ADAM17 (10-12). Modifications also extend the effective range of Shh functionality and are required for the development of protein gradients important in tissue morphogenesis (9, 13). Canonical signaling of Shh is mediated by a multicomponent receptor complex that includes Patched (PTCH1, PTCH2) and Smoothened (SMO) (14). The binding of Shh to PTCH releases the basal repression of SMO by PTCH. Shh activity can also be regulated through interactions with heparin, glypicans, and membrane-associated Hip (hedgehog interacting protein) (13, 15, 16).

References:

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12. Dierker, T. *et al.* (2009) *J. Biol. Chem.* **284**:8013.
13. Lewis, P.M. *et al.* (2001) *Cell* **105**:599.
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MANUFACTURING SPECIFICATIONS

GMP Proteins

R&D Systems, a Bio-Techne Brand's GMP proteins are produced according to relevant sections of the following documents: WHO TRS, No. 822, 1992 Annex 1, Good Manufacturing Practices for Biological Products; USP Chapter 1043, Ancillary Materials for Cell, Gene and Tissue-Engineered Products and USP Chapter 92, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.

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- Equipment calibration schedules using a computerized calibration program
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- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial bioburden testing (using broth culture, Sabourand's dextrose and blood agar plates with results reported at 3 days and at 7 days)
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

Additional testing and documentation requested by the customer can be arranged at an additional cost. Testing may include, but is not limited to, USP< 61> bioburden testing, positive identity testing, testing for adventitious agents and testing for residual host cell content.

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