Recombinant Human Sonic Hedgehog/Shh N-Terminus GMP

Catalog Number: 1314-GMP

DESCRIPTION

Source	*E. coli*-derived human Sonic Hedgehog/Shh protein Cys24-Gly197, with a C-terminal 6-His tag Accession # Q15465.1 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidelines.
N-terminal Sequence Analysis	(Cys_{24})-Gly-Pro-Gly-Arg-Gly-Phe-Gly-Lys-Arg
Predicted Molecular Mass	20 kDa

SPECIFICATIONS

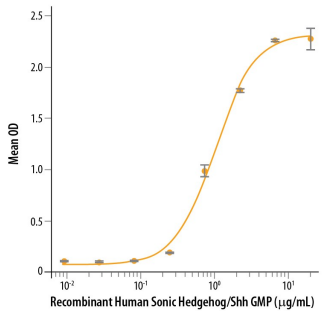
SDS-PAGE	22 kDa, reducing conditions
Activity	Measured by its ability to induce alkaline phosphatase production by C3H10T1/2 mouse embryonic fibroblast cells. Nakamura, T. *et al.* (1997) Biochem. Biophys. Res. Commun. **237**:465. The ED_{50} for this effect is <5 µg/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE with silver staining, under reducing conditions.
Mass Spectrometry	Intact mass analysis of recombinant human sonic hedgehog confirms the predicted molecular mass of 20425 Da.
Host Cell Protein	<0.5 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative for Mycoplasma.
Host Cell DNA	<0.0015 ng per µg of protein when tested by PCR.
Formulation	Lyophilized from a 0.2 µm filtered solution in NaH_2PO_4, NaCl and DTT . See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 250 µg/mL in sterile deionized water.
Shipping	The product is shipped with polar packs. 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.

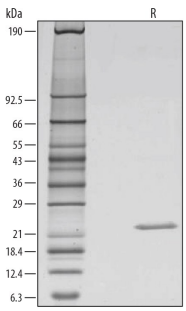
DATA

Bioactivity



Recombinant Human Sonic Hedgehog/Shh, N-Terminus GMP Bioactivity GMP-grade Recombinant Human Sonic Hedgehog/Shh, N-Terminus (Catalog # 1314-GMP) induces alkaline phosphatase production by the C3H10T1/2 mouse embryonic fibroblast cell line. The ED_{50} for this effect is <5 ug/mL.

SDS-PAGE



Recombinant Human Sonic Hedgehog/Shh, N-Terminus GMP SDS-PAGE 1 µg/lane of GMP-grade Recombinant Human Sonic Hedgehog/Shh, N-Terminus (Catalog # 1314-GMP) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a single band at 22 kDa.

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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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