Recombinant Human Sonic Hedgehog/Shh High Activity

Catalog Number: 8908-SH

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived Cys24-Gly197 Accession # Q15465
N-terminal Sequence Analysis	Cys24
Structure / Form	Cholesterol-modified at the C-terminal and fatty acid-modified at the N-terminal
Predicted Molecular Mass	20 kDa

SPECIFICATIONS

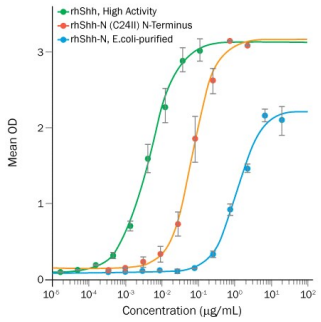
SDS-PAGE	18-24 kDa, reducing conditions
Activity	Measured by its ability to induce alkaline phosphatase production by C3H10T1/2 mouse embryonic fibroblast cells. Nakamura, T. *et al.* (1997) Biochem. Biophys. Res. Commun. **237**:465. The ED_{50} for this effect is typically 6-36 ng/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES, NaCl and CHAPS with BSA as a carrier protein. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

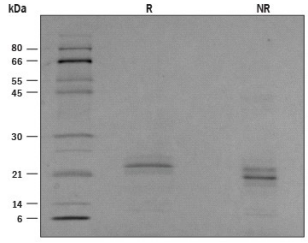
DATA

Bioactivity



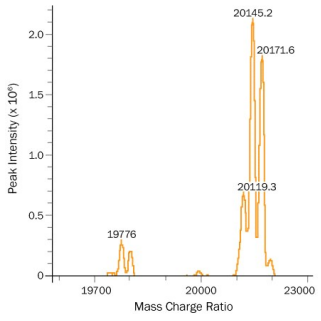
Enhanced Activity of Human Cell-expressed Sonic Hedgehog (Shh). Recombinant Human Shh proteins induce alkaline phosphatase production by mesenchymal stem cells. High Activity Shh (green), purified from HEK293 cells and containing the correct post-translational modifications (cholesterol and fatty acids), is over 14-fold more active than E. coli-purified Recombinant Human Shh-N (C24II) N-Terminus (Catalog # 1845-SH; red line), and over 250-fold more active than E. coli-purified Recombinant Human Shh-N (Catalog # 1314-SH; blue line).

SDS-PAGE



1 µg/lane of Recombinant Human Sonic Hedgehog/Shh was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing 18-24 kDa bands.

Mass Spectrometry



Post-translational Modification Analysis of Naturally-modified Recombinant Human Sonic Hedgehog (Shh). LC/ESI-MS analysis of Recombinant Human (rh)SHH, High Activity shows major peaks at 20119.3, 20145.2, and 20171.6 Da, suggesting that recombinant human SHH molecules are dual-modified with cholesterol at C-terminus, and fatty acids (lauric acid, myristic acid, and palmitic acid) at the N-terminus. The minor peaks at 19776 Da corresponds to rhSHH with only fatty acid modification.

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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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