

**DESCRIPTION**

<b>Source</b>	<i>E. coli</i> -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.
<b>N-terminal Sequence Analysis</b>	Met-Ile-Ile-Gly <sub>25</sub> -Pro-Gly-Arg-Gly-Phe-Gly
<b>Predicted Molecular Mass</b>	20 kDa

**SPECIFICATIONS**

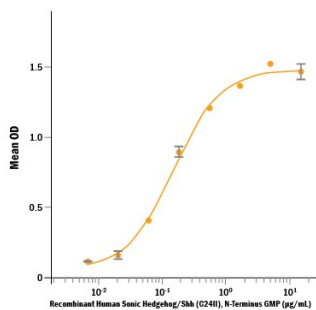
<b>SDS-PAGE</b>	22 kDa, reducing conditions
<b>Activity</b>	Measured by its ability to induce alkaline phosphatase production by C3H10T1/2 mouse embryonic fibroblast cells. Nakamura, T. <i>et al.</i> (1997) <i>Biochem. Biophys. Res. Commun.</i> <b>237</b> :465. The ED <sub>50</sub> for this effect is 0.1-0.4 µg/mL.
<b>Endotoxin Level</b>	<0.01 EU per 1 µg of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE with silver staining, under reducing conditions.
<b>Host Cell Protein</b>	<0.5 ng per µg of protein when tested by ELISA.
<b>Mycoplasma</b>	Negative when tested in a ribosomal RNA hybridization assay.
<b>Host Cell DNA</b>	<0.0015 ng per µg of protein when tested by PCR.
<b>Formulation</b>	Lyophilized from a 0.2 µm filtered solution in PBS and NaCl. See Certificate of Analysis for details.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 100-200 µg/mL in PBS, and allow up to 24 hours at 2 to 8 °C for complete reconstitution.
<b>Shipping</b>	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>• A minimum of 12 months when stored at ≤ -20 °C as supplied. Refer to lot specific COA for the Use by Date.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 3 months, ≤ -20 °C under sterile conditions after reconstitution.</li> </ul>

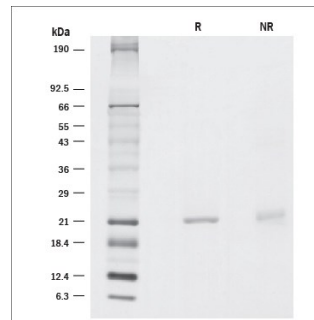
**DATA**

**Bioactivity**



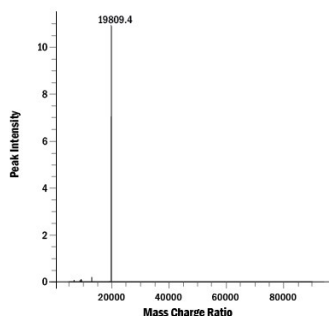
GMP-grade Recombinant Human Sonic Hedgehog/Shh (C24II), N-Terminus (Catalog # 1845-GMP) induces alkaline phosphatase production by the C3H10T1/2 mouse embryonic fibroblast cell line. The ED<sub>50</sub> for this effect is 0.1-0.4 µg/mL.

**SDS-PAGE**



1 µg/lane of GMP-grade Recombinant Human Sonic Hedgehog/Shh (C24II) N-Term (Catalog # 1845-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by silver staining, showing R band at 21.5 kDa and NR band at 22.5 kDa, respectively.

**Mass Spectrometry**



LC/ESI-MS analysis of GMP-grade Recombinant Human Sonic Hedgehog/Shh (C24II) N-Terminus (Catalog # 1845-GMP). The major peak at 19809 corresponds to the calculated molecular mass, 19814 Da.

**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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**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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- Raw material testing and vendor qualification/monitoring
- Fully validated equipment, processes and test methods
- Equipment calibration schedules using a computerized calibration program
- Facility maintenance, safety programs and pest control
- Material review process for variances
- Monitoring of stability over product shelf-life

R&D Systems strives to provide our customers with the analytical characteristics of each product so that customers may determine whether our products are appropriate for their research. The Certificate of Analysis provided contains the following lot specific information:

- N-terminal amino acid analysis, SDS-PAGE analysis, and endotoxin level (as determined by LAL assay) performed on each bulk QC lot, not on individual bottlings of each QC lot
- Post-bottling lot-specific bioassay results (compliance with an established range) and results of microbial testing according to USP
- Host Cell Protein testing performed by ELISA
- Mycoplasma testing by ribosomal RNA hybridization assay

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- Purified proteins are stored in animal-free containers in a dedicated cold storage room.

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- High quality product obtained under stringent conditions.

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