Recombinant Mouse Sonic Hedgehog/Shh GMP

Catalog Number: BT-SHH-GMP

DESCRIPTION

Source	*E. coli*-derived mouse Sonic Hedgehog/Shh protein Cys25 - Gly198 (Cys25Ile-Ile) with an N-terminal Met Accession # Q62226.2 Produced using non-animal reagents in an animal-free laboratory. Manufactured and tested under cGMP guidlines.
N-terminal Sequence Analysis	Met-Ile-Ile-Gly26-Pro-Gly-Arg-Gly-Phe-Gly
Predicted Molecular Mass	19.8 kDa

SPECIFICATIONS

SDS-PAGE	21 kDa, under 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 0.0500-0.600 µg/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>97%, by SDS-PAGE with quantitative densitometry by Coomassie® Blue Staining.
Mass Spectrometry	The molecular weight by mass spectrometry is 19844 Da ± 26 Da.
Host Cell Protein	<0.500 ng per µg of protein when tested by ELISA.
Mycoplasma	Negative for mycoplasma.
Host Cell DNA	<0.00150 ng per µg of protein when tested by PCR.
Formulation	Lyophilized from a 0.2 µm filtered solution in Sodium Phosphate, NaCl and DTT with Trehalose. See Certificate of Analysis for details.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 500 µg/mL in sterile 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.

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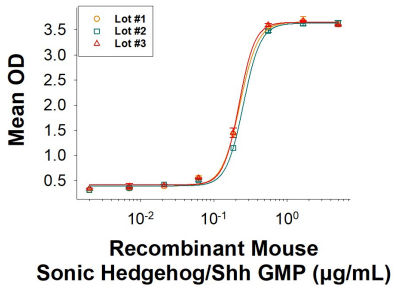
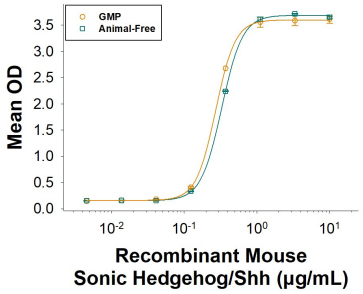
DATA

Bioactivity



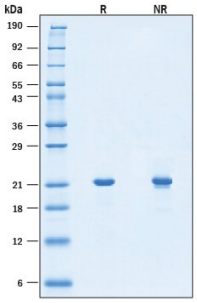
Recombinant Mouse Sonic Hedgehog/Shh GMP Protein Bioactivity. The bioactivity of Recombinant Mouse Sonic Hedgehog/Shh GMP Protein (Catalog # BT-SHH-GMP) was measured in the alkaline phosphatase production using C3H10T1/2 mouse embryonic fibroblast cells. Three independent lots were tested for bioactivity and plotted on the same graph to show lot-to-lot consistency of GMP Sonic Hedgehog/Shh protein.

Bioactivity



Equivalent Bioactivity of GMP and Animal-Free grades of Recombinant Mouse Sonic Hedgehog/Shh. Equivalent bioactivity of GMP (Catalog # BT-SHH-GMP) and Animal-Free (Catalog # BT-SHH-AFL) grades of Recombinant Mouse Sonic Hedgehog/Shh as measured in the alkaline phosphatase production using C3H10T1/2 mouse embryonic fibroblast cells (orange and green, respectively).

SDS-PAGE



Recombinant Mouse Sonic Hedgehog/Shh GMP Protein SDS-PAGE. 2 µg/lane of Recombinant Mouse Sonic Hedgehog/Shh GMP Protein (Catalog # BT-SHH-GMP) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at 21 kDa.

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). Mouse Shh encodes a 437 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:

1. Briscoe, J. and P.P. Therond (2013) Mol. Cell. Biol. **14**:416.
2. Aviles, E.C. *et al.* (2013) Front. Cell. Neurosci. **7**:86.
3. Xie, J. *et al.* (2013) OncoTargets Ther. **6**:1425.
4. Echelard, Y. *et al.* (1993) Cell **75**:1417.
5. Zeng, X. *et al.* (2001) Nature **411**:716.
6. Feng, J. *et al.* (2004) Development **131**:4357.
7. Goetz, J.A. *et al.* (2006) J. Biol. Chem. **281**:4087.
8. Pepinsky, R.B. *et al.* (1998) J. Biol. Chem. **273**:14037.
9. Chen, M.-H. *et al.* (2004) Genes Dev. **18**:641.
10. Etheridge, L.A. *et al.* (2010) Development **137**:133.
11. Jakobs, P. *et al.* (2014) J. Cell Sci. **127**:1726.
12. Dierker, T. *et al.* (2009) J. Biol. Chem. **284**:8013.
13. Lewis, P.M. *et al.* (2001) Cell **105**:599.
14. Carpenter, D. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:13630.
15. Filmus, J. and M. Capurro (2014) Matrix Biol. **35**:248.
16. Chuang, P.-T. and A.P. McMahon (1999) Nature **397**:617.

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