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Product Information

Monoclonal Anti-Sonic Hedgehog, N-terminal
Clone 171018
purified rat immunoglobulin

Catalog Number **S4944**

Product Description

Monoclonal Anti-Sonic Hedgehog (Shh), N-terminal (rat IgG2a isotype) is produced from the 171018 hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified, *E. coli*-derived, recombinant mouse Sonic Hedgehog N-terminal peptide (amino acids 25-198). The antibody is purified by Protein G affinity chromatography.

Monoclonal Anti-Sonic Hedgehog, N-terminal recognizes human and mouse sonic hedgehog, N-terminal peptide by immunoblotting, ELISA (capture), neutralization, and immunohistochemistry. In immunoblotting, the antibody shows approximately 50% cross-reactivity with the N-terminal peptide from recombinant mouse Desert hedgehog (Dhh; amino acids 23-198). The antibody shows no cross-reactivity with recombinant mouse Indian hedgehog (Ihh; amino acid 66-240) and no cross-reactivity with the C-terminal peptides of mouse Dhh (amino acids 199-396) or mouse Shh (amino acids 199-437).

Sonic Hedgehog (Shh) is an important cell signaling molecule expressed during embryonic development. Shh is involved in the patterning of the developing embryonic nervous system, somite, and limb. The N-terminal peptide of Shh is released by autoproteolysis and functions through interactions with a multicomponent receptor complex containing the transmembrane proteins, Patched and Smoothened. Shh protein is expressed in key embryonic tissues such as Hensen's node, the zone of polarizing activity in the posterior limb bud, the notochord, and the floor plate of the neural tube. Downstream targets of Shh include the transcription factors Gli3, responsible for Greigs polycephalosyndactyly in humans, and Hoxd13, responsible for polysyndactyly.[2-4]

Reagent

Lyophilized from 0.2 μm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 ml of 0.2 μm-filtered phosphate buffered saline to produce a 0.5 mg/ml stock solution of antibody.

Storage/Stability

Prior to reconstitution, store at –20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Avoid repeated freezing and thawing. Do not store in frost-free freezer.

Product Profile

Capture ELISA: this antibody can be used as a capture antibody (4 μg/ml) in a human or mouse Shh ELISA in combination with a detection antibody (biotinylated monoclonal Shh). Using plates coated with 100 μl/well of the capture antibody at 4 μg/ml, in combination with 100 μl/well of the detection antibody, an ELISA for sample volumes of 100 μl can be obtained. To arrive at the optimal dose range for this ELISA, a two-fold dilution series of the protein standard is set up starting with 1 ng/ml.

Neutralization: the antibody will neutralize the bioactivity of mouse Shh. The Neutralization $Dose_{50}$ (ND_{50}) is the concentration of antibody required to yield one-half maximal inhibition of the cytokine activity on a responsive cell line, when the cytokine is present at a concentration just high enough to elicit a maximum response. The exact concentration of antibody required to neutralize recombinant mouse Shh activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

Immunoblotting: a working antibody concentration of 1-2 μg/ml is recommended. The detection limit for recombinant mouse Shh N-terminal peptide is approximately 50 ng/lane under non-reducing and reducing conditions.

Immunohistochemistry: a working antibody concentration of approximately 25 μg/ml with a biotin conjugate detects Shh in frozen mouse embryo sections.

Note: In order to obtain the best results in various techniques and preparations, determination of optimal working dilutions by titration test is recommended.

Endotoxin: <0.1 EU (endotoxin units)/μg antibody as determined by the LAL (Limulus amebocyte lysate) method.

References

1. Echelard, Y. et al., Sonic hedgehog, a member of a family of putative signaling molecules, is implicated in the regulation of CNS polarity. Cell **75**, 1417-1430 (1993).
2. Perrimon, N., Hedgehog and beyond. Cell, **80**, 517-520 (1995).
3. Weed, M. et al., The role of sonic hedgehog in vertebrate development. Matrix Biol., **16**, 53-58 (1997).
4. Carpenter, D. et al., Characterization of two patched receptors for the vertebrate hedgehog protein family. Proc. Natl. Acad. Sci., **95**, 13630-13634 (1998).

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