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## Product Information

### Monoclonal Anti-Sonic Hedgehog, N-terminal

Clone 171018

Purified Rat Immunoglobulin

Product Number **S 4944**

#### 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 show 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 acid 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 multi-component receptor complex containing the trans-membrane proteins, Patched and Smoothed. Shh protein is expressed in key embryonic tissues such as the Hensen's node, zone of polarizing activity in the posterior limb bud, notochord, and floor plate of the neural tube. Downstream targets of Shh include the transcription factors Gli3, responsible for Greigs polycephalo-syndactyly in humans, and Hoxd13, responsible for polysyndactyly.<sup>2-4</sup>

#### Reagent

Monoclonal Anti-Sonic Hedgehog, N-terminal is supplied as approximately 500 µg of antiserum lyophilized from a 0.2 µm filtered solution of phosphate buffered saline with 5% trehalose.

#### 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

For 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 100µl/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.

For neutralization, the antibody will neutralize the bioactivity of mouse Shh. The Neutralization Dose<sub>50</sub> (ND<sub>50</sub>) 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.

For 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.

For 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, we recommend determining the optimal working concentrations by titration.

Endotoxin: < 0.1 EU (endotoxin units)/µg antibody as determined by the LAL (Limulus amoebocyte 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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