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RABBIT ANTI-HUMAN PROSURFACTANT PROTEIN B (proSP-B) POLYCLONAL ANTIBODY

CATALOG NO.: AB3430

LOT NUMBER:

QUANTITY: 100 µL

SPECIFICITY: Reacts strongly with human and mouse proSP-B (detects both the N-terminal and C-terminal propeptides). Does not react with mature SP-B. On human tissue immunostains alveolar Type II cells and non-ciliated bronchiolar cells. On mouse tissue immunostains alveolar Type II cells and non-ciliated bronchiolar (Clara cells) in distal and terminal airways.

IMMUNOGEN: Full length recombinant peptide (381 a.a.) of the human SP-B (pre)proprotein.

APPLICATIONS: Immunohistochemistry: 1:4,000-1:8,000 on human tissue with and without antigen retrieval (see protocol on back) and 1:1,000-1:2,000 on adult and fetal mouse lung (see notes on back).
Immunoblot: 1:1,000-1:5,000
ELISA: 1:500-1:1,000
Optimal dilutions must be determined by the end user.

SPECIES REACTIVITY: Human and mouse.

FORMAT: Rabbit serum.

PRESENTATION: Liquid. Contains no preservative.

STORAGE/HANDLING: Maintain at -20ºC in undiluted aliquots for up to twelve months. Avoid repeated freeze/thaw cycles.

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IMMUNOHISTOCHEMISTRY FOR AB3430

IMMUNOHISTOCHEMISTRY

Note: The enzymatic reaction product was enhanced with nickel cobalt to give a black precipitate.

Sections for antigen retrieval were immersed in sodium citrate buffer, pH 6.0, and heated in a microwave for 15 minutes at 90°C. Endogenous peroxidase was quenched for 15 min. at RT with 3% H_2O_2 in methanol. Sections were blocked with 2% normal goat serum in PBS with 0.2% Triton for 2 hours at RT before incubating overnight at 4°C with the properly diluted AB3430. The sections were then washed 6 times, 5 min. each, in PBS with 0.2% Triton and then incubated for 30 min. at RT with biotinylated goat anti-rabbit antibody (such as Chemicon catalog number AP132B) properly diluted in the blocking solution. Sections were incubated with the ABC complex diluted in blocking solution for 30 min. at RT; NiDAB in 0.1M acetate buffer for 4 min. at RT; Tris Cobalt for 4 min. at RT; and counterstained with nuclear fast red for 2 min. at RT. Controls included (1) formalin-fixed, paraffin-embedded, surgical samples of human pediatric and adult lung immunostained at the recommended dilutions or age-matched controls from wild-type, FVB/N, fetal and embryonic mouse pups and (2) omission of the AB3430 to check for endogenous biotin and peroxidase activity, as well as nonspecific binding of the secondary antibody.

Notes:
1) Dilutions used are for normal (i.e. uninjured) adult mouse lung and have been used on FVB/N, Balb/c, nu/nu and SCID mice. The use of antigen retrieval systems to enhance immunostaining for this antibody is not necessary in the mouse lung and may actually increase background staining.

2) Immunostaining of Clara cells for SP-B and proSP-B in the adult mouse lung can be variable from sample to sample. Most often when immunostaining is present it is seen in the most distal and terminal airways in the lung. It is specific for Clara cells and does not stain ciliated cells. It has not been found in the adult mouse tracheal cells or glands. Balb/c mice and strains of mice derived from a Balb/c background, such as nu/nu mice of SCID mice, appear to have increased amounts of SP-B in the bronchiolar epithelium by immunohistochemistry. (FVB/N = NIH3 < Balb/c.nu/nu < 'American' CB.17 scid/scid < 'European' CB.17 scid/scid mice (Jennings, Am. J. Respir. Cell Mol. Biol. (1995) 13:297-306 and Zsengeller, Hum. Gene Ther. (1997) 8:1331-1344.)

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