

Anti-Prosurfactant Protein C (proSP-C)



Polyclonal Antibody

Cat. # AB3786

Lot # 3073288

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

pack size: 100 µL

Store at -20°C

Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IH, ELISA	H, M, R	IgG	N/A	Rb	Varies	NP_003009

Background

Pulmonary surfactant is a complex mixture of phospholipids and proteins secreted from airway epithelial cells (Type II cells) in alveoli which reduces the surface tension at the alveolar air liquid interface, providing alveolar stability necessary for normal ventilation. Four distinct proteins isolated from pulmonary surfactant are termed surfactant proteins A, B, C and D. SP A (28 - 36 kDa) and SP D (43 kDa) are collagenous carbohydrate binding proteins, whereas SP B (8 - 9 kDa) and SP C (4 kDa) are non collagenous hydrophobic proteins. SP C is a fragment of a much larger precursor protein of 21 kDa. The precursor contains an exceedingly hydrophobic region of 34 amino acids that comprises most of the mature SP C, and a unique poly-valine domain.

Presentation

Polyclonal rabbit serum containing 0.01% sodium azide to preserve.

Specificity

Reacts strongly with proSP-C from human and mouse. Immunoprecipitates the 21 kDa proprotein as well as processing intermediates of M.W. 26 kDa, 21 kDa, 16 kDa and 14 kDa. Preincubation of the antiserum with the fusion protein results in ablation of immunostaining. On human tissue immunostains alveolar Type II cells in normal lung. The antibody also recognizes the aberrant form of proSP-C (6-12 kDa peptide) found in BAL and in alveolar proteinosis material on sections of lung samples from human patients with hereditary SP-B deficiency. On mouse tissue immunostains alveolar Type II cells in normal lung.

Species Cross-reactivity

Mouse and rat. Expected to cross-react with human.

Immunogen

A synthetic peptide corresponding to amino acids 1-32 from the N-terminal of the human SP-C proprotein coupled to KLH.

Molecular Weight

21 kDa proprotein as well as processing intermediates of M.W. 26 kDa, 21 kDa, 16 kDa and 14 kDa.

Storage and Handling

Maintain at -20°C in undiluted aliquots for up to twelve months after date of receipt. Avoid repeated freeze/thaw cycles.

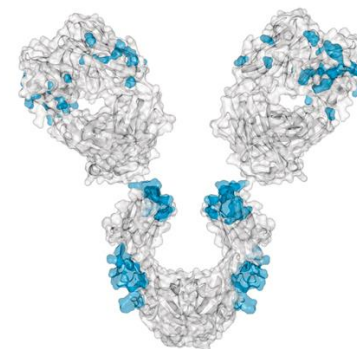
Control

Mouse lung epithelial cells.

Quality Control Testing

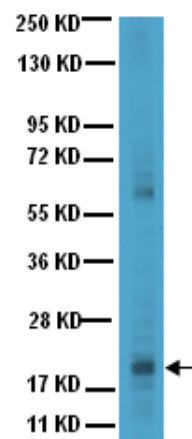
Routinely evaluated by Western Blot on rat lung lysates.

Western Blot Analysis: 1:500 dilution of this lot detected Prosurfactant Protein C on 10 ug of rat lung lysates.



References

1. Nogee, L.M., et al. (2000). *Am. J. Respir. Crit. Care Med.* 161:973-981.
2. Tokieda, K., et al. (1999). *Am. J. Respir. Cell and Mol. Biol.* 21:463-472.
3. Morotti, R.A., et al. (1999). *Human Pathology.* 30:618-625.
4. Zsengeller, Z.K., et al. (1997). *Hum. Gene Ther.* 8:1331-1344.
5. Jain-Vora, S., et al. (1997). *Am. J. Respir. Cell Mol. Biol.* 17:1-11.



Western Blot Analysis:
Rat lung lysate was resolved by electrophoresis, transferred to PVDF membrane and probed with anti-Prosurfactant Protein C (1:500 dilution of this lot). Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein Prosurfactant Protein C (~21 kDa).

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence

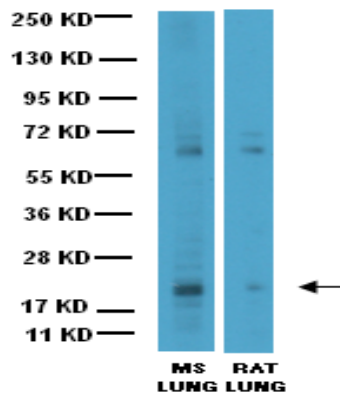
IH Immunohistochemistry (Tissue) ELISA Enzyme-linked Immunosorbent Assay

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications



Western Blot Analysis:

Representative images from a previous lot.

Mouse lung (Lane 1) and rat lung (Lane 2) were resolved by electrophoresis, transferred to PVDF membrane and probed with anti-Prosurfactant Protein C (1:500 dilution of a previous lot).

Proteins were visualized using a donkey anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein **Prosurfactant Protein C** (~21 kDa).

Immunohistochemistry: 1:2,000-1:4,000 dilution of a previous lot was used on human tissue (see protocol), 1:4,000 dilution on adult mouse lung and 1:1,000-1:2,000 dilution on fetal mouse lung (see protocol).

ELISA: 1:50,000 dilution of a previous lot was used on ELISA.

Optimal dilutions must be determined by the end user.

PROTOCOL

Western Blot

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA with 0.05% Tween[®]-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-Prosurfactant Protein C diluted in freshly prepared 5% BSA for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348 1:1000 dilution was used) in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use Spray and Glow Catalog # 17-373 to visualize results. Use as directed.

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

Endogenous peroxidase was quenched for 15 min. at RT with 3% H₂O₂ in methanol. Sections were blocked with 4% normal goat serum in PBS with 0.2% Triton for 2 hours at RT before incubating overnight at 4°C with the properly diluted Prosurfactant Protein C (AB3786). 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 Millipore 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 0.1 M 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 AB3786 to check for endogenous biotin and peroxidase activity, as well as nonspecific binding of the secondary antibody.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

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ANTI-PROSURFACTANT PROTEIN C (proSP-C)Cat # AB3786
Lot # 3073288**RELATED PRODUCTS (specific)**

cat #	description
AB3428	■ Anti-Prosurfactant Protein C
12-348	■ Goat anti-Rabbit HRP conjugated IgG
AP132B	■ Goat anti-Rabbit Biotinylated IgG
AP182P	■ Donkey anti-Rabbit HRP conjugated IgG.

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
64-202	■ Visualizer™ Western Blot Detection Kit, rabbit
DAB050	■ IHC Select® HRP/DAB Detection Kit
20774	■ IHC Select® Streptavidin Horseradish Peroxidase (HRP) Conjugate

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