

Detection of Prosurfactant Protein C in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Trypsin](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: ImmPRESS Anti-Rabbit Ig (peroxidase) Polymer Detection Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # MP-7401

Note: This kit contains a pre-dilute blocking serum and polymer reagent.

Primary Antibody: Rabbit Polyclonal Prosurfactant Protein C Antibody

Millipore / Chemicon International

Billerica, Massachusetts 01821

www.millipore.com

1-800-645-5476

Catalog # AB3786

Lot # 2087721

Negative Control Serum: Normal Rabbit Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 011-000-001

Staining Procedure

Positive Control Tissue: Lung

Stain Localization: Cytoplasmic (secreted) – Type II cells

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Proteolytic-Induced Epitope Retrieval Using Trypsin

Incubate the slides in a **0.1% trypsin** solution in a water bath at 37°C for 20 minutes.

(DO NOT add the trypsin to the 0.05M Tris-HCl • CaCl₂ solution until 5 minutes prior to incubation.

Trypsin loses 75% of its reactivity within 30 minutes at 37°C.)

Rinse the slides in distilled water for 1 minute to stop the enzymatic digestion.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

6. Block with the 2.5% normal horse serum from the ImmPRESS kit for 20 minutes at room temperature.

Lot # _____ Exp. Date _____

**DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BLOCK.**

7. Apply primary antibody at a 1:3000 dilution. Incubate for 1 hour at room temperature.

Lot # _____ Exp. Date _____

For negative control slides, dilute normal rabbit serum so that its protein concentration matches that of the primary antibody (if necessary). Then make a 1:3000 dilution. Apply the negative and incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

9. Apply the anti-rabbit polymer from the ImmPRESS kit, and incubate for 30 minutes at room temperature.

Lot # _____ Exp. Date _____

10. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

11. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.

(Add 1 drop of DAB per ml of substrate)

Lot # _____ Exp. Date _____ New Kit: yes / no

12. Rinse the slides in tap water 3 minutes.
13. Counterstain with hematoxylin for 20 seconds.
14. Rinse the slides in tap water until water is clear.
15. Gently agitate slides in 1X wash buffer until the tissues turn blue.
16. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

17. Coverslip

Updated 12/12/13