

# Detection of SP-A in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Trypsin](#)

[Normal Goat IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

### Blocking Serum: Normal Horse Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 008-000-001

### Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

### Primary Antibody: Goat Polyclonal SP-A Antibody (C-20)

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

[www.scbt.com](http://www.scbt.com)

1-800-457-3801

Catalog # sc-7699

### Secondary Antibody: Biotinylated Horse Anti-Goat IgG (H+L)

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # BA-9500

### Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # PK-7100

## Staining Procedure

Positive Control Tissue: Bronchiolar epithelium, type II cells  
Stain Localization: Cytoplasmic

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 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 • CaCl<sub>2</sub> 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 10% normal horse serum for 20 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no  
Apply avidin block for 15 minutes at room temperature.  
Quick rinse in 1X wash buffer.  
Apply biotin block for 15 minutes at room temperature.

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

8. Apply primary antibody at a 1:50 dilution. Incubate for 1 hour at room temperature.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute normal goat IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:50 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.
10. Apply the horse anti-goat secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.
12. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.  
Exp. Date \_\_\_\_\_ New Kit: yes / no
13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.
14. 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
15. Rinse the slides in tap water 3 minutes.
16. Counterstain with hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X wash buffer until the tissues turn blue.
19. 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

20. Coverslip

*Updated 10/15/10*