

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

## Reagents:

[1X Automation Buffer](#)  
[3% Hydrogen Peroxide](#)  
[Antibody Diluent](#)  
[Citrate Buffer](#)  
[DAB Chromagen](#)  
[Hematoxylin](#)

## Antibody Information

Blocking Serum: Normal Donkey Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Cat# 017-000-001

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: Rabbit anti-SP-A (H-148)  
Santa Cruz Biotechnology, Inc.  
Santa Cruz, CA 95060  
[www.scbt.com](http://www.scbt.com)  
1-800-457-3801  
Catalog #sc-13977

Negative Serum Control: Normal Rabbit Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #011-000-001

Secondary antibody: Biotinylated Donkey anti-rabbit  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #711-065-152

Label antibody: Vector Elite ABC  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #PK-6101

### **Staining Procedure**

- Positive Control Tissue: bronchiolar epithelium
- Stain Localization: cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Perform Heat Induced Epitope Retrieval using a microwave oven  
Unmasking Techniques  
Place a full rack of slides in a container containing 200mls of 1X citrate buffer.  
Microwave for 5 minutes at level 5.  
Cool for 1 minute  
Microwave again for 5 minutes at level 5. Temp. \_\_\_\_\_  
Remove the slides from the microwave oven and allow to cool 20 minutes at room temperature.  
Rinse slides in distilled water for 2 minutes. Repeat two times.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.

5. Block with 10% Normal Donkey Serum for 20 minutes at room temperature.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

6. Apply Avidin/Biotin block

Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Rabbit anti-SP-A) at 1:10 dilution and incubate for 1hr at room temperature.

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

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody (SP-A) and use this to make the 1:10 dilution. Apply normal rabbit serum to the slides and incubate for 1hr at room temperature.

Lot# \_\_\_\_\_ Reconstitution Date \_\_\_\_\_

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody (biotinylated donkey anti-rabbit) at 1:500 dilution and incubate for 30 minutes at room temperature.

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

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Vector Elite label and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use)

Exp. Date \_\_\_\_\_ New Kit: yes / no

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.  
(Add 1 drop of DAB per ml of substrate)

Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip.

updated 10/12/05