

# Detection of Mucin 2 in Formalin-Fixed, Paraffin-Embedded Human Tissue

## Reagents

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

## Antibody Information

Kit: Santa Cruz Rabbit ABC Staining kit

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

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

1-800-457-3801

Catalog #sc-2018

Note: This kit contains all reagents necessary to make blocking solution, secondary and label antibodies.

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 Polyclonal Mucin 2 (H-300) Antibody

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

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

1-800-457-3801

Catalog #sc-15334

Negative control serum: 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

## Staining Procedure

Positive Control Tissue: Human colon

Stain Localization: Cytoplasm of the goblet cells

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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. Unmasking Techniques using the decloaker.  
Add 500 ml D/W to the pan of the decloaker.  
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.  
Decloak for 5 min. Pressure \_\_\_\_\_  
Depressurize for 10 min.  
Remove pan top and cool for 10 min. Temp \_\_\_\_\_  
Rinse in D/W, 2x for 3 min each
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Block with Normal Goat Serum (1.5%) for one hour at room temperature.  
Made with 75ul normal goat serum in 5ml diluent  
Kit Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_
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.  
Wipe excess block  
  
**DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.**
7. Apply primary antibody (Mucin 2) at a 1:500 dilution and incubate for one hour at room temperature.  
Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (Mucin 2) and use this to make a 1:500 dilution and incubate for one hour at room temperature.

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

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
9. Apply secondary antibody (Biotinylated goat anti-rabbit IgG) and incubate for 30 minutes. Made with 75ul of normal goat serum and 25ul of goat anti-rabbit in 5ml of diluent.
10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
11. Apply label complex and incubate for 30 minutes. (Prepare at least 30 minutes prior to use.) Made with 50ul of avidin (A) and 50ul of biotinylated HRP (B) in 2.5ml of diluent.
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 20 seconds.
16. Rinse in tap water until water is clear.
17. Gently agitate slides in 1X Automation buffer until they turn blue.
18. Dehydrate through the following solutions.

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

19. Coverslip

*Updated 10/17/06*