

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Staining Kit: Rabbit ABC Staining System

Santa Cruz Biotechnology, Inc.

Santa Cruz, CA 95060

www.scbt.com

1-800-457-3801

Catalog # sc-2018

Note: This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-457-3801

Catalog # sc-15334

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: Colon (goblet cells)
Stain Localization: Cytoplasm

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. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* _____

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each time.

Rabbit ABC Staining Kit

Lot # _____ Exp. Date (1 year from received date) _____

6. Apply the blocking reagent from the Goat Staining Kit for 1 hour at room temperature.
(Made with 75 µl goat serum (blue cap) and 5 ml diluent)

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 SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
ONLY WIPE EXCESS BUFFER.

8. Apply the primary antibody at a 1:500 dilution, and incubate for 1 hour at room temperature.
Lot # _____ Date Aliquoted _____

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:500 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

Lot # _____ Date Reconstituted _____

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
10. Apply the secondary antibody from the Rabbit Staining Kit, and incubate for 30 minutes at room temperature. (Made with 75 μ l goat serum, 25 μ l of goat anti-rabbit IgG (green cap), and 5 ml diluent)
11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
12. Apply the label complex from the Rabbit Staining Kit, and incubate for 30 minutes at room temperature. (Made with 50 μ l reagent A (white cap), 50 μ l reagent B (purple cap), and 2.5 ml diluent)
13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
14. Apply the DAB chromagen, and 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 Harris Hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X Wash Buffer until they turn blue.
19. Dehydrate through the following solutions:

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

20. Coverslip

Updated 10/17/06