

Detection of Smooth Muscle Actin in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen peroxide](#)

[Antibody Diluent](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information:

Block: Protein Block Serum-Free--Ready to use

Dako Corporation

Carpinteria CA

www.dakousa.com

1-800-235-5763

Catalog # X0909

Primary antibody: EPOS Smooth Muscle Actin

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Catalog #U7033

Negative antibody: EPOS Negative control

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Catalog # U0951

Staining Procedure

Positive Control Tissue: Smooth muscle in female reproductive organs or GI tract

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 5 minutes.
2. Rinse slides in 1X Automation Buffer for 5 minutes each.
3. Unmasking Technique using the decloaker.
Add 500ml of distilled water to the pan of the decloaker.
Place a full rack of slides in a Tissue Tek™ container containing 250ml of 1X citrate buffer solution.
Decloak for 5 minutes. Pressure _____
Depressurize for 10 minutes.
Remove pan top and cool for 10 minutes. Temperature _____
Rinse in distilled water two times for 3 minutes each
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Block with Dako Serum-free Protein Block for 10 minutes at room temperature.
Lot# _____ Exp Date _____

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

6. Incubate sections using prediluted antibodies for 30 minutes at room temperature.
EPOS Smooth Muscle Actin Lot # _____ Exp. Date _____
EPOS Negative Control Lot # _____ Exp. Date _____
7. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
8. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
(Add 1 drop of DAB per ml of substrate)
Lot # _____ Exp date: _____

9. Rinse in tap water 3 minutes.

10. Counterstain with Modified Harris Hematoxylin for 30 seconds.

11. Rinse in tap water until water is clear.

12. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

13. Dehydrate through the following solutions.

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

14. Coverslip

updated 05/24/06