

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1% BSA Diluent](#)

[Normal Rabbit IgG – Affinity Purified](#)

[ProLong Gold Mounting Media](#)

Blocking Serum: Normal Goat Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog # 005-000-121

Primary Antibody: Rabbit Polyclonal Antibody to Alpha Smooth Muscle Actin

Abcam, Inc

Cambridge, MA 02139

www.abcam.com

1-888-772-2226

Catalog # ab5694

Secondary Antibody: Alexa Fluor® 568 Goat Anti-Rabbit IgG (H+L) *Highly Cross-Adsorbed*

Life Technologies / Invitrogen

Grand Island, NY 14072

www.invitrogen.com

1-888-584-8929

Catalog # A-11036

Staining Procedure

Positive Control Tissue: Tissue with smooth muscle – e.g. female reproductive and gastrointestinal tracts
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 the slides in 2 changes of 1X wash buffer for 5 minutes each.
4. Block with 10% normal goat serum for 20 minutes at room temperature.
Lot # _____ Date Reconstituted _____

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

5. Apply the primary antibody at a 1:500 dilution. Incubate for 1 hour at room temperature.
Lot # _____ Exp. Date _____

For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 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 _____

6. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

From this point on, please perform the stain in the dark.

7. Apply the Alexa 568 goat anti-rabbit secondary antibody at a 1:300 dilution. Incubate for 30 minutes at room temperature.
Lot # _____ Exp. Date _____
8. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
9. Rinse the slides in tap water.
10. Coverslip with ProLong Gold Mounting Media (with or without DAPI).
11. Store slides at 4°C

Updated 11/11/11