www.biossusa.com
800.501.7654 [DOMESTIC]
+1.781.569.5821 [INT'L]
support@biossusa.com

Mouse Alpha smooth muscle Actin Ready-To-Use IHC Kit

Cat. No.: IHC0114M

Size: 50T (including a control slide)
Sample Type: FFPE tissue
Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

General Information

Number	Component	50T	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Mouse Alpha smooth muscle Actin Mouse mAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Mouse IgG pAb)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	-20℃
9	Counter Staining Reagent	5 ml	RTU	RT
10	Differentiation Reagent	6 ml	RTU	RT
11	Mounting Media	5 ml	RTU	RT
12	Control slide (Mouse colon)	1 slide	RTU	RT
13	Datasheet	1 copy		

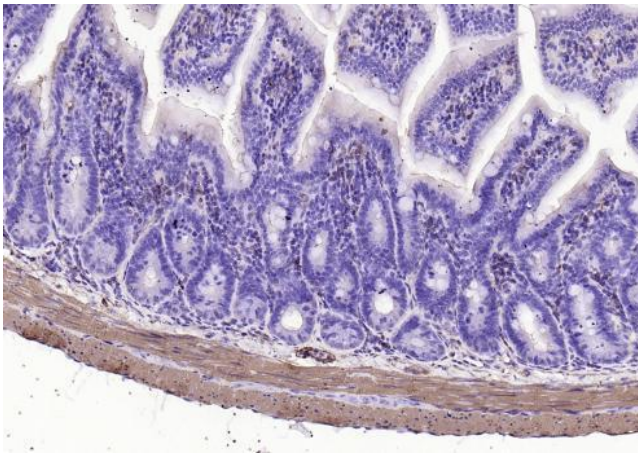
Background

Smooth Muscle Actin belongs to the actin family of proteins, which are highly conserved proteins that play a role in cell motility, structure and integrity. Alpha, beta and gamma actin isoforms have been identified, with alpha actin being a major constituent of the contractile apparatus, while beta and gamma actins are involved in the regulation of cell motility. In particular, smooth muscle actin is an alpha actin that is found in skeletal muscle. Actin exists as a ubiquitous protein involved with filament formation that make up large portions of the cytoskeleton. Actin filaments interact with myosin to assist in muscle contraction as well as aiding in cell motility and cytokinesis. Smooth muscle actin is found on smooth muscle vessel walls, gut wall, myometrium, myoepithelial cells in breast and salivary glands. Defects in the smooth muscle actin gene cause aortic aneurysm familial thoracic type 6. Actin isoforms differ slightly in their N-terminus and the sequences of each are perfectly conserved in higher vertebrates. Alpha-smooth muscle actin is abundant in vascular and visceral smooth muscle cells. In addition, it has also been shown that smooth muscle actin appear in stress fibers of fibroblastic cells during pathological situations involving contractile phenomena such as wound healing and fibrocontractive diseases. Multiple alternatively spliced variants of smooth muscle actin have been identified.

Synonyms

alpha sarcomeric Actin; alpha smooth muscle Actin; Actin alpha; ASMA; ASM-A; alpha-SMA; alpha SMA; AAT6; ACTA2; Actin alpha 2 smooth muscle aorta; Actin aortic smooth muscle; ACTSA; ACTVS; Alpha 2 actin; Alpha-actin 2; Cell growth inhibiting gene 46 protein; Growth inhibiting gene 46; ACTA_MOUSE; Actin alpha 2 smooth muscle aorta; Actin aortic smooth muscle; Actin, aortic smooth muscle; Alpha 2 actin; Alpha actin 2; Alpha cardiac actin; Alpha-actin 2; Alpha-actin-2; Cell growth inhibiting gene 46 protein; Cell growth-inhibiting gene 46 protein; Growth inhibiting gene 46; MYMY5.

Validation Data



Immunohistochemical analysis of paraffin embedded mouse colon tissue slide using IHC0114M (Mouse Alpha smooth muscle Actin IHC Kit).

Immunohistochemistry Protocol

1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen Retrieval

Add 100×**Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

3. Block Endogenous Peroxidase

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

4. Serum Blocking

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Mouse Alpha smooth muscle Actin Mouse mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

6. Secondary Antibody Incubation

Incubate slides with 2-4 drops of **HRP-Goat anti-Mouse IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

7. Signal Development

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

8. **Counterstain**

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of differentiation reagent to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. Dehydration Sheet

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4℃ after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0114M, Bioss Antibodies". Citation example: "Mouse tissue sections using Mouse Alpha smooth muscle Actin IHC Kit (IHC0114M, Bioss Antibodies) were stained for Alpha smooth muscle Actin according to the manufacturer's instructions."