



Actin, Smooth-Muscle

IHC of Actin, Smooth-Muscle on an FFPE Appendix Tissue

Description Actin is a major component of the cytoskeleton and is present in every cell type. Actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells. In vertebrates 3 main groups of actin isoforms (alpha, beta and gamma) have been identified. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. The beta and gamma actins coexist in most cell types as components of the cytoskeleton and as mediators of internal cell motility.

Smooth-Muscle Actin antibody does not stain cardiac or skeletal muscle; however, it will stain myofibroblasts and myoepithelial cells. This antibody could be used together with Muscle-Specific Actin to distinguish Leiomyosarcoma from Rhabdomyosarcoma. In most cases of Rhabdomyosarcoma, this antibody gives negative results whereas M. S. Actin is positive in the rhabdomyoblasts. Leiomyosarcomas are positive with both M. S. Actin and S. M. Actin antibodies.

Antibody Type	Mouse Monoclonal	Clone	ASM/H12
Isotype	IgG2a/K	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Appendix, Uterus
Storage	Store at 2°-8°C	Stability	2 years

For long-term storage of the concentrated antibody, it is recommended that aliquots of the antibody be frozen at -20°C in glycerol 50% (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Dilute using an antibody diluent such as ImmunoDetector Protein Block/Antibody Diluent (BSB 0040 and BSB 0041) or ImmunoDNA Background Blocker (BSB 0103-BSB 0107).

Presentation S. M. Actin is a mouse monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Availability	Catalog No.	Antibody Type	Dilution	Volume/QTY
	BSB 5029	Prediluted	Ready-To-Use	3.0 ml
	BSB 5030	Prediluted	Ready-To-Use	7.0 ml
	BSB 5031	Prediluted	Ready-To-Use	15.0 ml
	BSB 5032	Concentrated	1:50-1:250	0.1 ml
	BSB 5033	Concentrated	1:50-1:250	0.5 ml
	BSB 5034	Concentrated	1:50-1:250	1.0 ml
	BSB 5035	Control Slides		5

Note: For concentrated antibodies, please centrifuge prior to use to ensure recovery of all product.

- References**
1. Cooke PH, *J Cell Biol.* 1976;68:539-556
 2. Skalli O, et al. *J Cell Biol.* 1986;103:2787-2796
 3. Gown AM, et al. *J Cell Biol.* 1985;100:807-813
 4. Kuroda M, *Biochem Biophys Acta.* 1985;843:20-213
 5. Lazarides E, *J Histochem Cytochem.* 1975;223:507-52

Protocol Suggested protocol on reverse

Recommended Immunohistochemical Protocol

- Pretreatment**
1. Cut and mount 3-4 micron formalin-fixed paraffin-embedded tissues on positive charged slides.
 2. Air dry for 2 hours at 58° C.
 3. Deparaffinize, dehydrate and rehydrate tissues.
 4. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as **ImmunoDNA Retriever with Citrate** (BSB 0020-BSB 0023) or **EDTA** (BSB 0030-BSB 0033).
 5. Any of three heating methods may be used:
 - a. **Electric Pressure Cooker**
Place standoff rack at base of pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high, and incubate for 15 minutes. Open and immediately transfer slides to room temperature.
 - b. **Water Bath Method**
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the **ImmunoDNA Retriever with Citrate** or **EDTA** in a water bath set at 95°-99° C. Incubate for 30-60 minutes.
 - c. **Conventional Steamer Method**
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the **ImmunoDNA Retriever with Citrate** or **EDTA** in a Steamer, cover and steam for 30-60 minutes.
 6. After heat treatment, transfer slides in **ImmunoDNA Retriever with Citrate** or **EDTA** to room temperature and let stand for 15-20 minutes.
 7. Wash slides with IHC wash buffer or DI water.
 8. Continue IHC staining protocol.

Immunohistochemical Protocol

Step	ImmunoDetector (AP or HRP)	PolyDetector (AP or HRP)
Peroxidase/AP Block	5 minutes	5 minutes
Primary Antibody	30 minutes	45 minutes
Secondary Biotinylated Link	10 minutes	Not Applicable
AP or HRP Label	10 minutes	45 minutes
Substrate-Chromogen	5-10 minutes	10 minutes
Counterstaining	Time varies with counterstain	Time varies with counterstain

