Human/Mouse/Rat α-Smooth Muscle Actin Antibody

Monoclonal Mouse IgG_{2A} Clone # 1A4

Catalog Number: MAB1420

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human α-Smooth Muscle Actin.
Source	Monoclonal Mouse IgG_{2A} Clone # 1A4
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Peptide corresponding to residues EEEDSTALVC of the N-term of human α-smooth muscle actin (1)
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

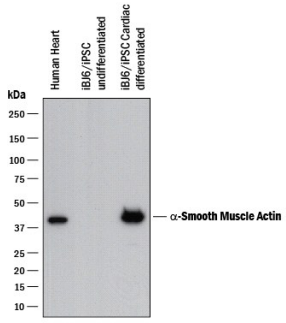
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1-0.25 µg/mL	See Below
Immunocytochemistry	8-25 µg/mL	See Below
Immunohistochemistry	8-25 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Simple Western	10 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

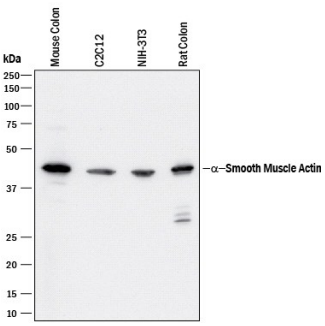
DATA

Western Blot



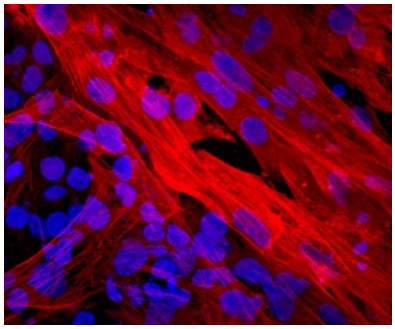
Detection of Human α-Smooth Muscle Actin by Western Blot. Western blot shows lysates of human heart tissue and iBJ6 human induced pluripotent stem cell line undifferentiated or cardiac differentiated. PVDF membrane was probed with 0.25 µg/mL of Mouse Anti-Human/Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for α-Smooth Muscle Actin at approximately 40 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



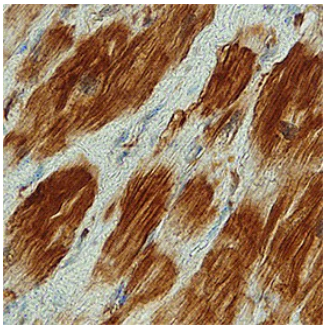
Detection of Mouse and Rat α-Smooth Muscle Actin by Western Blot. Western blot shows lysates of mouse colon tissue, C2C12 mouse myoblast cell line, NIH-3T3 mouse embryonic fibroblast cell line, and rat colon tissue. PVDF membrane was probed with 0.1 µg/mL of Mouse Anti-Human/Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for α-Smooth Muscle Actin at approximately 42 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



α-Smooth Muscle Actin in BG01V Human Embryonic Stem Cells. α-Smooth Muscle Actin was detected in immersion fixed BG01V human embryonic stem cells differentiated to cardiomyocytes using Mouse Anti-Human/Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and cytoskeleton. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



α-Smooth Muscle Actin in Human Heart. α-Smooth Muscle Actin was detected in immersion fixed paraffin-embedded sections of human heart using Mouse Anti-Human/Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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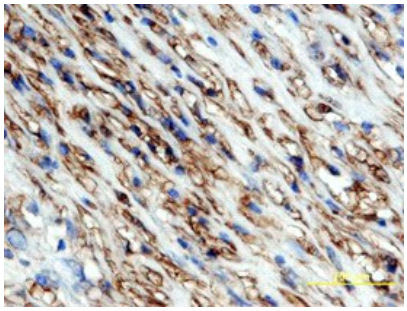
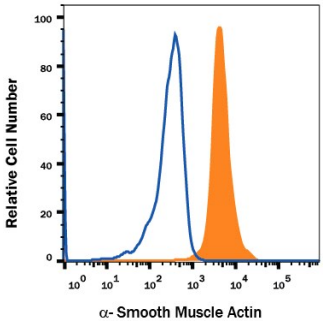
Immunohistochemistry



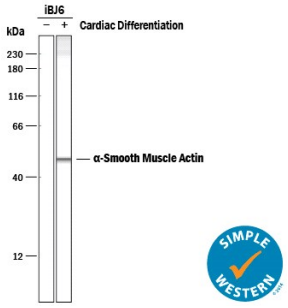
α-Smooth Muscle Actin in Human Breast Cancer Tissue. α-Smooth Muscle Actin was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Mouse Anti-Human/ Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420) at 8 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Intracellular Staining by Flow Cytometry



Detection of α-Smooth Muscle Actin in Human Blood Monocytes by Flow Cytometry. Human peripheral blood monocytes were stained with Mouse Anti-Human/Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420, filled histogram) or isotype control antibody (Catalog # MAB003, open histogram), followed by Allophycocyanin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0101B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for Staining Intracellular Molecules.

Simple Western



Detection of Human α-Smooth Muscle Actin by Simple Western™. Simple Western lane view shows lysates of iBJ6 human induced pluripotent stem cell line untreated (-) or differentiated to cardiomyocytes (+), loaded at 0.2 mg/mL. A specific band was detected for α-Smooth Muscle Actin at approximately 49 kDa (as indicated) using 10 µg/mL of Mouse Anti-Human/Mouse/Rat α-Smooth Muscle Actin Monoclonal Antibody (Catalog # MAB1420) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

α-Smooth Muscle Actin has been frequently used as a marker of smooth muscle differentiation (1, 2).

References:

1. Skalli, O. *et al.* (1986) J. Cell Biol. **103**:2787.
2. Oishi, K. *et al.* (2002) J. Physiol. **540**:139.

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