Human/Mouse Smad2/3 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3797

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human, mouse, and rat Smad2/3 in Western blots. Predicted to detect rat based on sequence homology.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human Smad3 Ser2-Ala230 Accession # P84022
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 either lyophilized or as a 0.2 μm filtered solution in PBS.

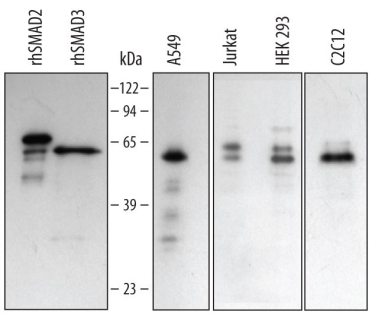
APPLICATIONS

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	0.5 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/5 x 10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	3-15 µg/mL	Immersion fixed paraffin-embedded sections of human brain (cortex and cerebellum)
Simple Western	25 µg/mL	See Below

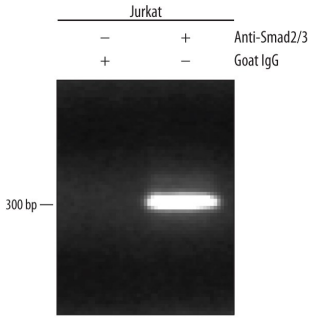
DATA

Western Blot



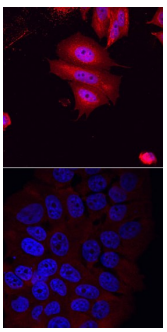
Detection of Human/Mouse Smad2/3 by Western Blot. Western blot shows lysates of A549 human lung carcinoma cell line, Jurkat human acute T cell leukemia cell line, HEK293 human embryonic kidney cell line, and C2C12 mouse myoblast cell line. PVDF membrane was probed with 0.5 µg/mL Goat Anti-Human/Mouse Smad2/3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3797) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). For additional reference, recombinant human Smad2 and Smad3 were included. Specific bands for Smad2 were detected at approximately 64 and 58 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 2.

Chromatin Immunoprecipitation (ChIP)



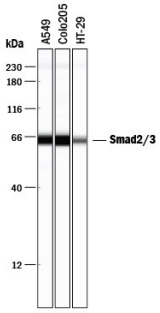
Detection of Smad2/3-regulated Genes by Chromatin Immunoprecipitation. Jurkat human acute T cell leukemia cell line treated with 50 ng/mL PMA and 200 ng/mL calcium ionomycin for 30 minutes was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Smad2/3/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human/Mouse Smad2/3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3797) or control antibody (Catalog # Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCellect Streptavidin Ferrofluid (Catalog # Catalog # MAG999) and DNA was purified using chelating resin solution. The *c-myc* promoter was detected by standard PCR.

Immunocytochemistry



Smad2/3 in MCF-7 Human Cell Line. Smad2/3 was detected in immersion fixed MCF-7 human breast cancer cell line induced (upper panel) or non-induced (lower panel) to undergo epithelial-mesenchymal transition (EMT) using Goat Anti-Human/Mouse Smad2/3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3797) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm and, in EMT-induced cells, nuclei. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Simple Western



Detection of Human Smad2/3 by Simple Western™. Simple Western lane view shows lysates of A549 human lung carcinoma cell line, COLO 205 human colorectal adenocarcinoma cell line, and HT-29 human colon adenocarcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for Smad2/3 at approximately 64 kDa (as indicated) using 25 µg/mL of Goat Anti-Human/Mouse Smad2/3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3797) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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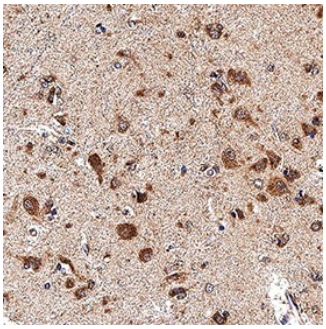
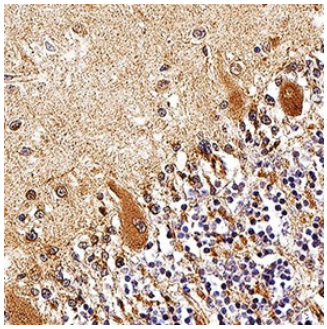
Immunohistochemistry



Smad2/3 in Human Brain (Cortex). Smad2/3 was detected in immersion fixed paraffin-embedded sections of human brain (cortex) using Goat Anti-Human/Mouse Smad2/3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3797) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). 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 DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in neurons. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



Smad2/3 in Human Brain (Cerebellum). Smad2/3 was detected in immersion fixed paraffin-embedded sections of human brain (cerebellum) using Goat Anti-Human/Mouse Smad2/3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3797) at 10 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). 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 DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in Purkinje neurons. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 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

Smads are a family of intracellular proteins that transmit transforming growth factor beta (TGF-β) superfamily signals from the cell surface to the nucleus. The Smad family is divided into three subclasses: receptor regulated Smads, (Smads 1, 2, 3, 5 and 8); the common partner, (Smad4); and the inhibitory Smads, (Smads 6 and 7). The binding of TGF-β or activin to their cognate receptor induces phosphorylation of Smads 2 and 3. The activated Smads associate with the common-mediator subunit, Smad4, and the heteromeric complex translocates into the nucleus to initiate transcription. Smad3, also known as Mothers Against Decapentaplegic homolog 3 (MADH3), shares 83% amino acid identity with Smad2, also known as Mothers Against Decapentaplegic homolog 2 (MADH2). Human Smad2 has 99% identity to mouse and rat Smad2. Human Smad3 has 99% identity to mouse and rat Smad3.

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