

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human Smad4 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 15% cross-reactivity with recombinant human (rh) Smad1 and less than 5% cross-reactivity with rhSmad9, rhSmad5, and rhSmad7 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Smad4 Pro139-Asp332 Accession # Q13485
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.

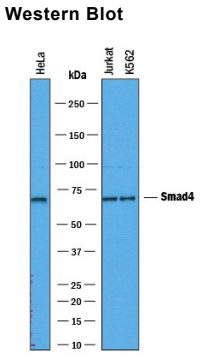
APPLICATIONS

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	0.25 µg/mL	See Below
Chromatin Immunoprecipitation (ChIP)	5 µg/5 x 10 ⁶ cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below

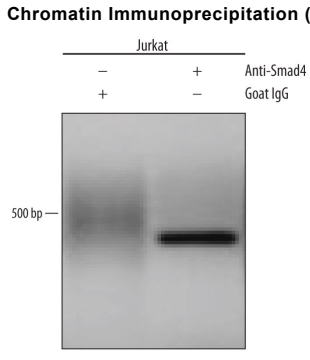
DATA

Western Blot



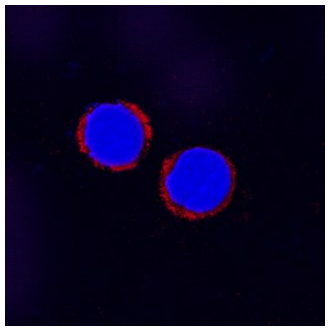
Detection of Human Smad4 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, Jurkat human acute T cell leukemia cell line, and K562 human chronic myelogenous leukemia cell line. PVDF membrane was probed with 0.25 µg/mL of Goat Anti-Human Smad4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2097) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for Smad4 at approximately 60 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

Chromatin Immunoprecipitation (ChIP)



Detection of Smad4-regulated Genes by Chromatin Immunoprecipitation. Jurkat human acute T cell leukemia cell line treated with 10 ng/mL Recombinant Human IL-12 (Catalog # 219-IL) overnight was fixed using formaldehyde, resuspended in lysis buffer, and sonicated to shear chromatin. Smad4/DNA complexes were immunoprecipitated using 5 µg Goat Anti-Human Smad4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2097) or control antibody (Catalog # AB-108-C) for 15 minutes in an ultrasonic bath, followed by Biotinylated Anti-Goat IgG Secondary Antibody (Catalog # BAF109). Immunocomplexes were captured using 50 µL of MagCelect Streptavidin Ferrofluid (Catalog # MAG999) and DNA was purified using chelating resin solution. The *p21* promoter was detected by standard PCR.

Immunocytochemistry



Smad4 in Human PBMCs. Smad4 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Goat Anti-Human Smad4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2097) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

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. <ul style="list-style-type: none"> • 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. Upon signal-induced phosphorylation, Smad subunits associate with the common-mediator subunit, Smad4. This heteromeric complex then translocates into the nucleus to exert transcriptional comodulator activity.