

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human Smad9 in direct ELISAs. In direct ELISAs, approximately 5% cross-reactivity with recombinant human Smad1 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Smad9 Ala158-Ser223 Accession # O15198
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.

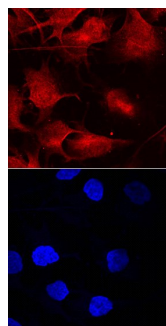
APPLICATIONS

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
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

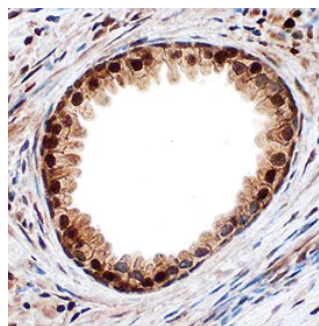
DATA

Immunocytochemistry



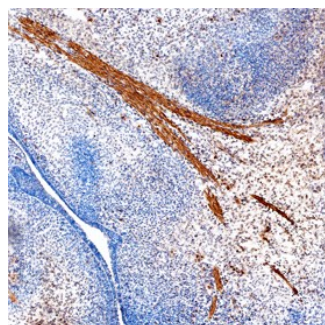
Smad9 in A172 Human Cell Line. Smad9 was detected in immersion fixed A172 human glioblastoma cell line using Sheep Anti-Human/Mouse Smad9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7215) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red, upper panel; Catalog # NL010) and counterstained with DAPI (blue, lower panel). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Smad9 in Human Prostate Cancer Tissue. Smad9 was detected in immersion fixed paraffin-embedded sections of human prostate cancer tissue using Sheep Anti-Human Smad9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7215) at 3 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-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to nuclei in glandular epithelial cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Immunohistochemistry



Smad9 in Mouse Embryo. Smad9 was detected in immersion fixed frozen sections of mouse embryo (13 d.p.c.) using Sheep Anti-Human Smad9 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7215) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to developing muscle cells. View our protocol for [Chromogenic IHC Staining of Frozen Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

SMAD9 (SMA protein and Mothers Against Decapentaplegic 9; also SMAD8 and MAHD6) is a 55-60 kDa R-Group member of the dwarfin/SMAD family of proteins. It is expressed by BMP-responsive cells, and serves to transmit information from the BMP receptor (BMPR) to the nucleus. In particular, following BMPR activation, SMAD9 is phosphorylated by the BMPR on its C-terminus, promoting heterodimerization with SMAD4 and its translocation into the nucleus. Here, SMAD9 binds to DNA and participates in gene activation. Human SMAD9 is 467 amino acids (aa) in length. It contains a DNA-binding domain termed MH1 (aa 16-140) plus a receptor/transcription factor binding domain termed MH2 (aa 273-467). The C-terminus contains two phosphorylation sites at Ser465 and Ser467. There is one splice form that shows a deletion of aa 224-260. Over aa 158-223, human and mouse SMAD9 share 82% identity in amino acid sequence.