Smad1/5 [pS463/pS465] ABfinity™ Recombinant Rabbit Monoclonal Antibody - Purified

Catalog no. 700047

(See product label for lot information)

Clone/PAD: 31H14L11
Isotype: IgG
Gene ID: 4086, 4090
Protein Acc. no.: Q15797, Q99717
Qty: 100 µg
Volume: 200 µl
Concentration: 0.5 mg/ml

Formulation
PBS + 0.09% azide

Immunogen
A peptide corresponding to amino acids 456-465 of Q15797 and Q99717.

Immunogen sequence
SPLNPIS[pS]V[pS]

Reactivity
This antibody reacts with human Smad1/5 [pS463/pS465]. Based on sequence identity and similarity, reactivity to mouse, rat, primates, and numerous other species is expected.

Specificity
This antibody is specific for Smad1/5 [pS463/pS465] and does not recognize non-phosphorylated Smad1/5.

Storage
2-8°C for up to 1 mo, -20°C for long term storage. Avoid repeated freezing and thawing.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Validated Applications:

	Species	Test Material	Concentration
Immunohistochemistry	human	lung, breast, and thyroid carcinoma	4-6 µg/ml
Immunofluorescence	human	HeLa	2-4 µg/ml
Flow Cytometry	human	Jurkat + BMP-4	0.5-1 µg/test

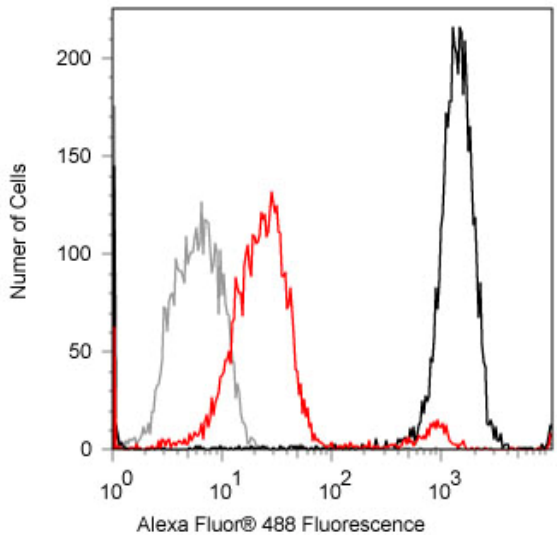
Background
Smad proteins are intracellular signal transducers and downstream effectors of TGF-β/BMP signaling. Three distinct classes of Smads have been defined: the receptor-regulated (R-Smads), which include Smad1, 2, 3, 5, 8; the common-mediator Smads (co-Smads), including Smad4, and the antagonistic or inhibitory Smads (I-Smads), including Smad6 and 7 (1). Smad1 (MADR1) mediates signals of bone morphogenetic proteins (BMPs) in the cell (2). BMPs play a role in ectopic bone formation and inductive interactions during the early development. BMPs constitute a large family of signaling molecules that regulate a wide range of critical processes including morphogenesis, cell-fate determination, proliferation, differentiation and apoptosis of both vertebrates and invertebrates (3,4). Phosphorylation of Smad1, a human homolog of MAD, is regulated and rapidly induced by bone morphogenic proteins (BMP) but not TGFβ or activin (5). BMP receptors are members of the TGFβ family of Ser/Thr kinase receptors, and ligand binding induces multimerization, autophosphorylation and activation of these receptors (6-8). Subsequently, they phosphorylate Smad1 at Ser 463 and Ser 465 in the C-terminal motif SSXS, as well as Smad5 and Smad8 at their corresponding sites. Signaling through the BMP-Smad 1/5/8 pathway has been implicated in embryonic morphogenesis (8). The dual phosphorylation site recognized by this antibody is S463/S465 in Smad1 (Accession Q15797) and Smad5 (Accession Q99717) and S465/S467 in Smad8 (Accession O15198).

References

1. Derynck R, et al. (1998) Smads: Transcriptional activators of TGF-β responses. Cell 95:737-740.
2. Liu F, et al. (1996) A human Mad protein acting as a BMP-regulated transcriptional activator. Nature 381:620-623.
3. Hogan BL. (1996) Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev 10:1580-1594.
4. Hoodless PA, et al. (1996) MADR1, a MAD-related protein that functions in BMP2 signaling pathways. Cell 85:489-500, 1996.
5. Macias-Silva M, et al. (1998) Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. J Biol Chem 273:25628-25636.
6. Klemm JD, et al. (1998) Dimerization as a regulatory mechanism in signal transduction. Annu Rev Immunol 16:569-592.
7. Kretzschmar M, et al. (1997) The TGF-beta family mediator Smad1 is phosphorylated directly and activated functionally by the BMP receptor kinase. Genes Dev 11:984-995.
8. Whitman M. (1998) Smads and early developmental signaling by the TGFβ superfamily. Genes Dev 12:2445-2462.
9. Eivers E, et al. (2008) Integrating positional information at the level of Smad1/5/8. Curr Opin Genet Dev.18(4):304-10.

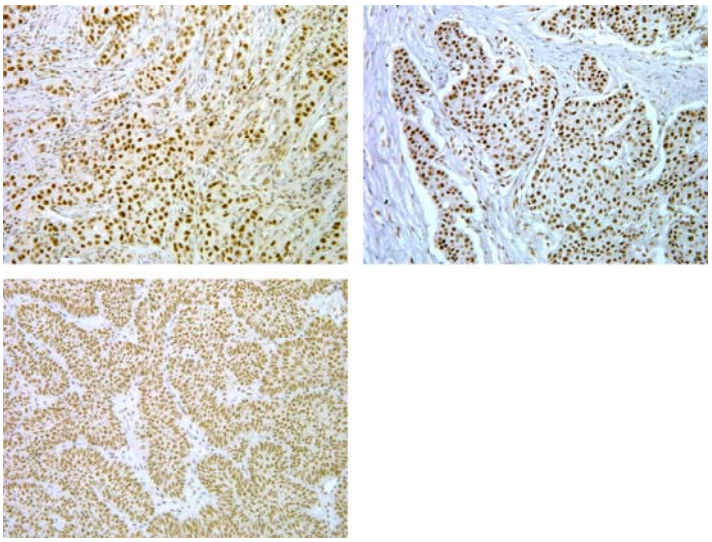
www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

This antibody is manufactured under a licensed process covered by Patent # 5, 599, 681.



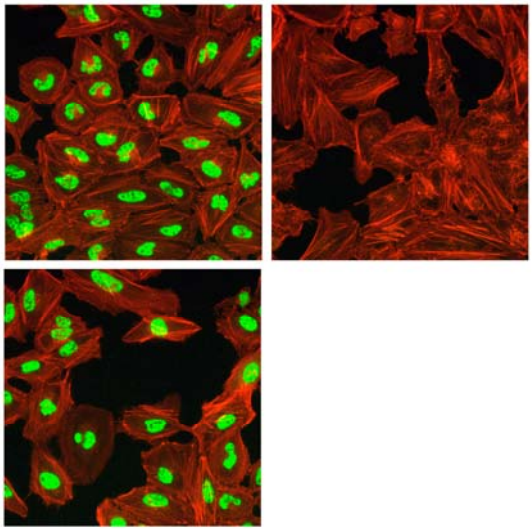
Flow cytometry of Jurkat cells labeled with rabbit anti-Smad1/5 [pS463/pS465] (Cat. No. 700047).

Jurkat cells were fixed and permeabilized using FIX & PERM® (Cat. No. GAS004) reagents. Cells were stimulated with (black trace) or without (gray trace) BMP-4 and stained with 0.5 µg anti-SMAD1/5 [pS463 / pS465] followed by Alexa Fluor® 488 goat anti-rabbit Ig (Cat. No. A11008). Pre-incubation with the immunogenic peptide decreased the signal (red trace).



Immunohistochemistry of human lung, breast and thyroid carcimona tissues labeled with rabbit anti-Smad1/5 [pS463/pS465] (Cat. No. 700047).

FFPE human lung (top left), breast (top right) and thyroid (bottom) carcinoma tissues were labeled with rabbit anti-Smad1/5 [pS463/pS465] (5 µg/ml). Tissues were pretreated with EDTA and detected with SuperPicTure™ Polymer DAB (Cat. No.87-8963). Images were taken at 20x magnification. Note nuclear staining in tumor cells.



Immunocytochemistry of HeLa cells labeled with rabbit anti-Smad1/5 [pS463/pS465] (Cat. No. 700047).

HeLa cells were labeled with rabbit anti-SMAD1/5 [pS463/pS465] (2.5 µg/ml) in the absence of peptides (top left), or the presence of phosphopeptide used as immunogen (top right) or non-phosphopeptide (bottom left). Alexa Fluor® 488 goat anti-rabbit (Cat. No. A11008) at 1:1000 was used as secondary antibody. Actin was stained with Alexa Fluor® 568 Phalloidin (Cat. No. A12380).