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Store at -20C
#9520

Phospho-SMAD3 (Ser423/425) (C25A9) Rabbit mAb

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, W-S, IP, ChIP	H M R	Endogenous	52	Rabbit IgG	#P84022	4088

Product Usage Information

For optimal ChIP results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 x 10⁶ cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:250
Immunoprecipitation	1:50
Chromatin IP	1:100

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

Specificity/Sensitivity

Phospho-SMAD3 (Ser423/425) (C25A9) Rabbit mAb detects endogenous levels of SMAD3 when phosphorylated at Ser423/425. This antibody does not cross-react with other family members.

Species predicted to react based on 100% sequence homology

Monkey, Xenopus, Zebrafish, Bovine

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser423/425 of human SMAD3.

Background

Members of the SMAD family of signal transduction molecules are components of a critical intracellular pathway that transmit TGF-β signals from the cell surface into the nucleus. Three distinct classes of SMADs have been defined: the receptor-regulated SMADs (R-SMADs), which include SMAD1, 2, 3, 5, and 9; the common-mediator SMAD (co-SMAD), SMAD4; and the antagonistic or inhibitory SMADs (I-SMADs), SMAD6 and 7 (1-5). Activated type I receptors associate with specific R-SMADs and phosphorylate them on a conserved carboxy-terminal SSXS motif. The phosphorylated R-SMADs dissociate from the receptor and form a heteromeric complex with SMAD4, initiating translocation of the heteromeric SMAD complex to the nucleus. Once in the nucleus, SMADs recruit a variety of DNA binding proteins that function to regulate transcriptional activity (6-8).

Following stimulation by TGF-β, Smad2 and Smad3 become phosphorylated at carboxyl terminal serine residues (Ser465 and 467 on Smad2; Ser423 and 425 on Smad3) by TGF-β Receptor I. Phosphorylated Smad 2/3 can complex with Smad4 and translocate to the nucleus to regulate gene expression (9-11).

Background References

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- Liu, X. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94, 10669-10674.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **W-S:** Simple Western™ **IP:** Immunoprecipitation **ChIP:** Chromatin IP

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat

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