abcam

Product datasheet

SMAD3 (pS423/S425) ELISA Kit ab186038

SimpleStep ELISA

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Overview

Product name SMAD3 (pS423/S425) ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
A431 extract	6			2.4%

Inter-assay

Sample	n	Mean	SD	CV%
A431	3			7.4%

Sample type Cell Lysate, Tissue Homogenate

Assay type Semi-quantitative

Sensitivity 10 µg/ml

Range 10 µg/ml - 500 µg/ml

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Mouse, Human

Predicted to work with: Rat

Product overview Abcam's SMAD3 (pS423/S425) *in vitro* SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the semi-quantitative measurement of SMAD3 (pS423/S425) protein in human and mouse cells.

The SimpleStep ELISA™ employs a labeled capture and detector antibody which immunocaptures the sample analyte in solution. This entire complex (capture antibody/protein/detector antibody) is in turn immobilized in the well by immunoaffinity via the anti-tag antibody. Samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material; the TMB substrate is then added. The reaction is stopped by addition of Stop Solution which stops the color development and

completes any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

ASSAY SPECIFICITY

The SMAD3 (pS423/S425) assay detects endogenous levels of SMAD3 (GenBank Accession NP_001138574) in cellular lysates, only when phosphorylated at Ser423/425. Based on sequence similarity, cross reaction to SMAD2 may occur.

SPECIES REACTIVITY

This kit detects SMAD3 (pS423/S425) in Human and mouse cell culture extracts. Detection in rat samples is also expected. Other species should be tested on a case-by-case basis.

Serum and plasma samples have not been tested with this kit.

Notes

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform Microplate

Properties

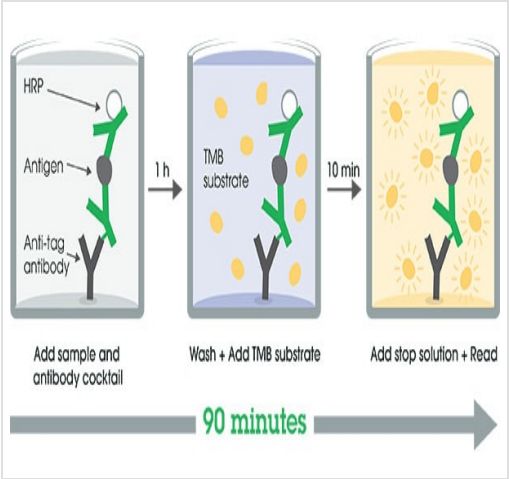
Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests	1 x 96 tests
10X Wash Buffer PT	1 x 15ml	1 x 15ml
50X Cell Extraction Enhancer Solution	1 x 1ml	1 x 1ml
5X Cell Extraction Buffer PTR	1 x 12ml	1 x 12ml
Lyophilized SMAD3 (pS423/S425) Control Lysate	1 vial	1 vial
Plate Seal	1 unit	1 unit
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	1 unit
SMAD3 (pS423/S425) Capture Antibody	1 x 3ml	1 x 3ml
SMAD3 (pS423/S425) Detector Antibody	1 x 3ml	1 x 3ml
Stop Solution	1 x 12ml	1 x 12ml
TMB Substrate	1 x 12ml	1 x 12ml

Function Receptor-regulated SMAD (R-SMAD) that is an intracellular signal transducer and transcriptional

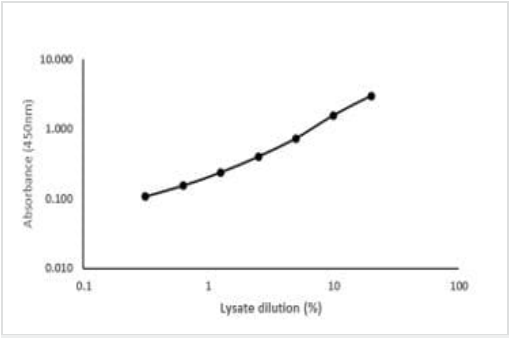
	modulator activated by TGF-beta (transforming growth factor) and activin type 1 receptor kinases. Binds the TRE element in the promoter region of many genes that are regulated by TGF-beta and, on formation of the SMAD3/SMAD4 complex, activates transcription. Also can form a SMAD3/SMAD4/JUN/FOS complex at the AP-1/SMAD site to regulate TGF-beta-mediated transcription. Has an inhibitory effect on wound healing probably by modulating both growth and migration of primary keratinocytes and by altering the TGF-mediated chemotaxis of monocytes. This effect on wound healing appears to be hormone-sensitive. Regulator of chondrogenesis and osteogenesis and inhibits early healing of bone fractures. Positively regulates PDPK1 kinase activity by stimulating its dissociation from the 14-3-3 protein YWHAQ which acts as a negative regulator.
Involvement in disease	Colorectal cancer Loeys-Dietz syndrome 3
Sequence similarities	Belongs to the dwarfin/SMAD family. Contains 1 MH1 (MAD homology 1) domain. Contains 1 MH2 (MAD homology 2) domain.
Domain	The MH1 domain is required for DNA binding. Also binds zinc ions which are necessary for the DNA binding. The MH2 domain is required for both homomeric and heteromeric interactions and for transcriptional regulation. Sufficient for nuclear import. The linker region is required for the TGFbeta-mediated transcriptional activity and acts synergistically with the MH2 domain.
Post-translational modifications	Phosphorylated on serine and threonine residues. Enhanced phosphorylation in the linker region on Thr-179, Ser-204 and Ser-208 on EGF and TGF-beta treatment. Ser-208 is the main site of MAPK-mediated phosphorylation. CDK-mediated phosphorylation occurs in a cell-cycle dependent manner and inhibits both the transcriptional activity and antiproliferative functions of SMAD3. This phosphorylation is inhibited by flavopiridol. Maximum phosphorylation at the G(1)/S junction. Also phosphorylated on serine residues in the C-terminal SXS motif by TGFBR1 and ACVR1. TGFBR1-mediated phosphorylation at these C-terminal sites is required for interaction with SMAD4, nuclear location and transactivational activity, and appears to be a prerequisite for the TGF-beta mediated phosphorylation in the linker region. Dephosphorylated in the C-terminal SXS motif by PPM1A. This dephosphorylation disrupts the interaction with SMAD4, promotes nuclear export and terminates TGF-beta-mediated signaling. Phosphorylation at Ser-418 by CSNK1G2/CK1 promotes ligand-dependent ubiquitination and subsequent proteasome degradation, thus inhibiting SMAD3-mediated TGF-beta responses. Phosphorylated by PDPK1. Acetylation in the nucleus by EP300 in the MH2 domain regulates positively its transcriptional activity and is enhanced by TGF-beta. Ubiquitinated. Monoubiquitinated, leading to prevent DNA-binding. Deubiquitination by USP15 alleviates inhibition and promotes activation of TGF-beta target genes. Poly-ADP-ribosylated by PARP1 and PARP2. ADP-ribosylation negatively regulates SMAD3 transcriptional responses during the course of TGF-beta signaling.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic and nuclear in the absence of TGF-beta. On TGF-beta stimulation, migrates to the nucleus when complexed with SMAD4 (PubMed:15799969). Through the action of the phosphatase PPM1A, released from the SMAD2/SMAD4 complex, and exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

Images



SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

Other - SMAD3 (pS423/S425) ELISA Kit (ab186038)



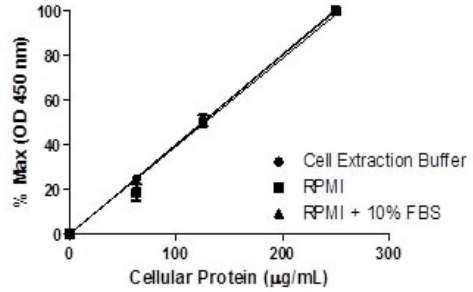
Raw data values are shown in the table.

SMAD3 (pS423/S425) control lysate dilution series in 1X Cell Extraction Buffer PTR.

Lysate Dilution Series Measurements			
Control Lysate (%)	O.D 450 nm		Mean O.D
	1	2	
0.000	0.054	0.061	0.058
0.31	0.107	0.106	0.107
0.63	0.143	0.167	0.155
1.25	0.235	0.241	0.238
2.5	0.405	0.396	0.401
5	0.719	0.747	0.733
10	1.564	1.576	1.570
20	2.972	2.990	2.981

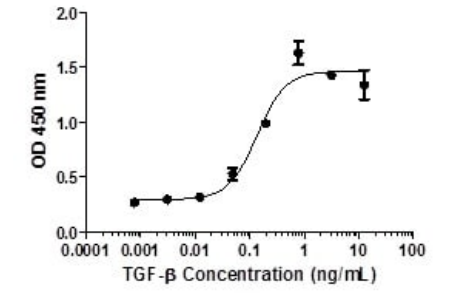
Example of a typical SMAD3 (pS423/S425) control lysate dilution series in 1X Cell Extraction Buffer PTR. The SMAD3 lysate dilution series was prepared as described. Raw data values are shown in the table.

SMAD3 (pS423/S425) control lysate dilution series in 1X Cell Extraction Buffer PTR.



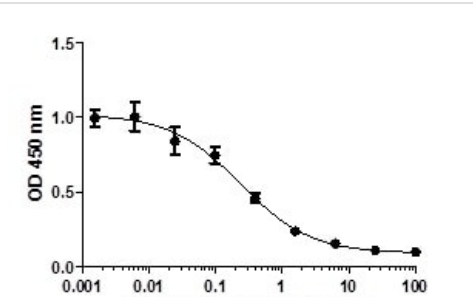
Linearity of dilution

Linearity of dilution in representative sample matrices. Cellular lysates were prepared at 3 concentrations in common media containing 1X Cell Extraction Buffer PTR. Data from duplicate measurements of SMAD3 (pS423/S425) are normalized and plotted.



SMAD3 (pS423/S425) phosphorylation in response to TGF-beta treatment.

Induction of SMAD3 (pS423/S425) phosphorylation in HeLa cells in response to TGF-β treatment. HeLa cells were cultured in 96-well tissue culture plates, serum-starved and treated (60 min) with a dose-range of TGF-β before cell lysis. Data from quadruplicate measurements of SMAD3 (pS423/S425) are plotted.



SMAD3 (pS423/S425) phosphorylation in response to SB431542 treatment.

Inhibition of SMAD3 (pS423/S425) phosphorylation in HeLa cells in response to SB431542 treatment. HeLa cells were cultured in 96-well tissue culture plates and treated with a dose-range of SB431542 (60 min). Cells were then stimulated with TGF-β (60 min) and lysed. Data from quadruplicate measurements of SMAD3 (pS423/S425) are plotted.

Lysate Dilution Series Measurements			
Control Lysate (%)	O.D 450 nm		Mean O.D
	1	2	
0.000	0.054	0.061	0.058
0.31	0.107	0.106	0.107
0.63	0.143	0.167	0.155
1.25	0.235	0.241	0.238
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5	0.719	0.747	0.733
10	1.564	1.576	1.570
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Example of a typical SMAD3 (pS423/S425) control lysate dilution series in 1X Cell Extraction Buffer PTR.

The SMAD3 lysate dilution series was prepared as described. Raw data values are shown in the table.

Kit Control lysates are provided at a concentration that give consistent signal between different lots. Lysates are produced and formulated by signal intensity to be consistent to within 30% of the previous lot. As such, Control lysates are not provided with a protein concentration.