abcam

Product datasheet

Human SMAD3 ELISA Kit ab264624

Recombinant SimpleStep ELISA

4 Images

Overview

Product name Human SMAD3 ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Extract	8			1.2%

Inter-assay

Sample	n	Mean	SD	CV%
Extract	3			1.5%

Sample type Cell Lysate

Assay type Sandwich (quantitative)

Sensitivity 55.72 pg/ml

Range 187.5 pg/ml - 12000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell Lysate	109	107% - 112%

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Human

Product overview Human SMAD3 ELISA Kit (ab264624) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of SMAD3 protein in cell lysate. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human SMAD3 with 55.72 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This

approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (**ab203359**) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

Notes

SMAD3 is a key protein involved in cytokine signaling and is essential for the proper regulation of proliferation, differentiation and apoptosis. Upon phosphorylation by TGF-b receptors, SMAD3 complexes with SMAD4 and upregulates goes controlled by SMAD-binding elements. SMAD3 is currently under investigation for its role in type 2 diabetes and cancer. The immunogen for this kit is an N-terminal fragment and shares high homology with many mammalian homologs.

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

Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions

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

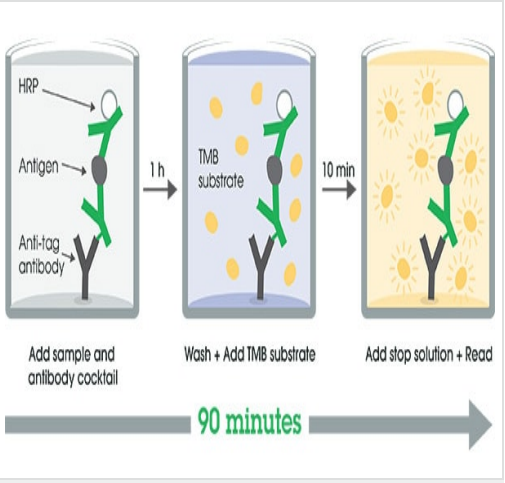
Components	1 x 96 tests
SMAD3 Lyophilized Recombinant Protein	2 vials
10X SMAD3 Capture Antibody	1 x 600µl
10X SMAD3 Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BI	1 x 6ml
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit

Components	1 x 96 tests
Stop Solution	1 x 12ml
TMB Development Solution	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

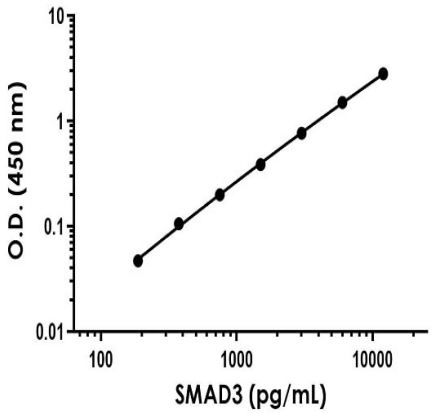
exported out of the nucleus by interaction with RANBP1 (PubMed:16751101, PubMed:19289081). Co-localizes with LEMD3 at the nucleus inner membrane (PubMed:15601644). MAPK-mediated phosphorylation appears to have no effect on nuclear import (PubMed:19218245). PDPK1 prevents its nuclear translocation in response to TGF-beta (PubMed:17327236).

Images



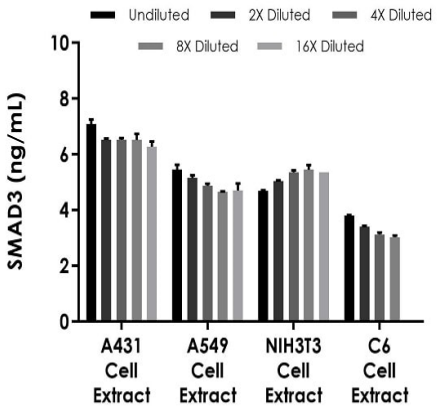
SimpleStep ELISA Protocol Diagram

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.



Example of SMAD3 standard curve in 1X Cell Extraction Buffer PTR.

The SMAD3 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



Interpolated concentrations of native SMAD3 in A431, A549, NIH3T3, and C6 cell extract samples based on a 250 µg/mL, 250 µg/mL, 250 µg/mL, and 125 µg/mL extract loads, respectively.

The concentrations of SMAD3 were measured in duplicate and interpolated from the SMAD3 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean SMAD3 concentration was determined to be 6.6 ng/mL in A431 cell extract samples, 5 ng/mL in A549 cell extract samples, 5.2 ng/mL in NIH3T3 cell extract samples, and 3.3 ng/mL in C6 cell extract samples.

Sandwich ELISA - Human SMAD3 ELISA Kit (ab264624)

To learn more about the advantages of recombinant antibodies see **here**.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors