

ELISAONE™ SMAD3 Assays

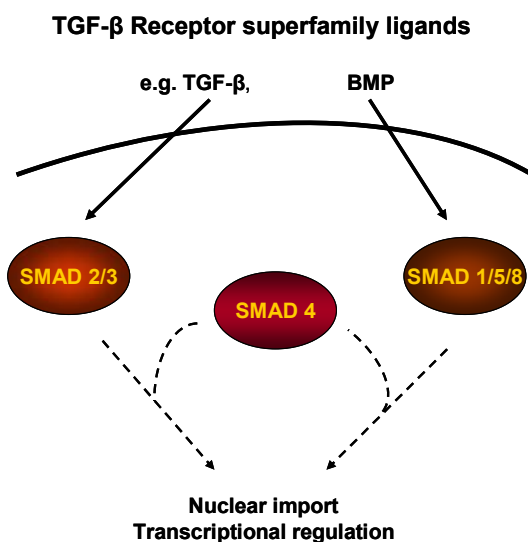
ELISAONE™ assay kits for analysis of cellular SMAD3 phosphorylation

ELISAONE™ Overview

ELISAONE assay kits are for the detection of cellular proteins. A whole new way of performing cellular assays, ELISAONE takes the hard work out of running a standard ELISA, while still giving the high quality results expected from a sandwich immunoassay. Fully self-contained kits are supplied in a convenient 96-well format. Simple to use and highly sensitive, ELISAONE kits are designed to get results, fast.

ELISAONE™ phospho-SMAD3 assays

Mothers against decapentaplegic homolog 3, also known as Smad3, is a member of the Smad family of proteins, which belong to the TGFβ superfamily of cell modulators. Smad3 is involved in cell signalling, and mediates signals of activin and TGFβ's for a range of cellular processes. TGFβ binding to the receptors induces activation of the receptors, which can then phosphorylate SMAD3 at Ser423 and Ser425, and Smad2 at the corresponding C-terminal site.



Activated SMADs dimerize with SMAD4, translocate to the nucleus, and stimulate transcription of particular genes. TGFβ-mediated signaling regulates several cellular processes.

ELISAONE™ SMAD3 (p-Ser423/425) assay technical specifications

Specificity:

TGR's ELISAONE SMAD3 (p-Ser423/425) assay kits detect endogenous levels of SMAD3 (GenBank Accession NP_001138574) in cellular lysates. Phospho-SMAD3 assay kits only detect SMAD3 when phosphorylated at Ser423/425. Based on sequence similarity, cross reaction to SMAD2 may occur.

Species cross-reactivity:

Tested: Human, mouse
Expected: Rat

Other species should be tested on a case-by-case basis.

Kit contents:

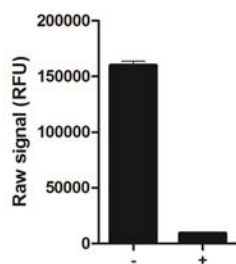
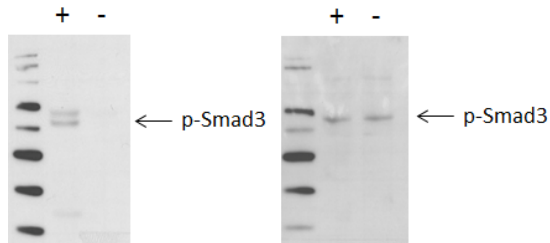
	24-pt Reagent kit	96-well full kit
Capture Antibody Reagent	1 x 0.75 mL	1 x 3.0 mL
Detection Antibody Reagent	1 x 0.75 mL	1 x 3.0 mL
Lysis Buffer (5X)	1 x 2 mL	1 x 8 mL
Enhancer Solution	1 x 0.5 mL	1 x 1 mL
ADHP Dilution Buffer	2 x 1.5 mL	2 x 6 mL
ADHP (100X)	1 x 30 µL	1 x 120 µL
Wash Buffer (10X)	1 x 2 mL	1 x 8 mL
Stop Solution	1 x 0.5 mL	1 x 2 mL
Assay Control Lysate	1 x 250 µL	1 x 250 µL
ELISA-One™ assay microplate	n/a	1 x 96-well microplate
Adherent plate seals	n/a	2

QC:

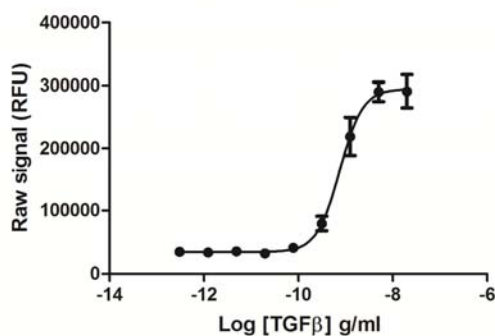
Phospho-SMAD3 assays are routinely tested against C2C12 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ p-SMAD3 assay performance

Using the p-SMAD3 assay kit or Western blot, SMAD3 phosphorylation at Ser423/425 is detected in TGF-treated C2C12 cells (+), compared with untreated C2C12 cells.



Raw264.7 cells were seeded at 80K cells/well in a 96 well tissue culture microplate overnight. The next day cells were serum starved for 60 minutes, then stimulated with various concentrations of TGFβ for 60 min. The medium was removed from the wells, and cells were lysed with 120 μl/well of Lysis Mix, with shaking for 10 min. The lysates were transferred to an ELISAONE assay plate and assayed for phospho-Smad3, using the standard protocol. Signal in the wells was determined using an Envision plate reader (PerkinElmer).



Ordering Information

ELISAONE Assay Kits are available now from:

CEDARLANE (www.cedarlanelabs.com)

US: 1-800-721-1644

CANADA: 1-800-268-5058

AXXORA (www.axxora.com)

US: 1-858-550-8830

Email : axxora-usa@axxora.com

Product	Pack size	Catalog
phospho-Smad 3 (S423/425)	24 assays	EKT028
	96 assays	ERS028

* ELISAONE assay microplates are not supplied with 24pt kits, but can be purchased separately.

Complementary Products

ELISAONE™ assay kits are available for the following targets:

MAPK Signaling:

ERK 1/2, p38 MAPK, JNK/SAPK, c-JUN

AKT Signaling:

AKT 1/2/3, p70S6K, RPS6, BAD

STAT Signaling:

STAT3, STAT5

NF-κB Signaling:

NF-κB p65, I-κBα, IKKα

Other targets:

p53, CREB1, SMAD1, SMAD3

Wnt Signaling:

β-Catenin, GSK3β

Protein normalization:

GAPDH

Also available separately:

Product	Pack Size	Catalog
ELISAONE™ 96-wellregular microplates	1 plate	EPF001
	5 plates	EPF002
ELISAONE™ 96-wellstrip-well microplates	1 plate	EPL001
	5 plates	EPL002
5X Lysis Buffer	100mL	EBF001
Enhancer Solution	100mL	EBF002
10X Wash Buffer	500mL	EBF003

Check our website (www.tgrbio.com) for the latest information on target availability.