

ELISAONE™ SMAD1 Assays

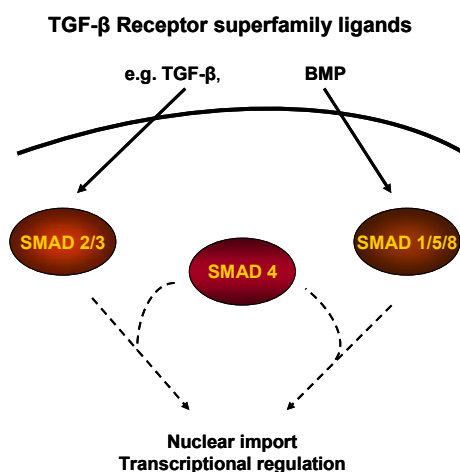
ELISAONE™ assay kits for analysis of cellular SMAD1 phosphorylation

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-SMAD1 and Total SMAD1 assays

Bone morphogenetic protein (BMPs) receptors belong to the TGFbeta-receptor superfamily, and bind BMPs to regulate a range of cellular processes, via the SMAD signaling pathway. BMP binding to the receptors induces multimerization and activation of the receptors, which can then phosphorylate SMAD1 at Ser463 and Ser465, and SMAD5 and SMAD8 at corresponding C-terminal sites.



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

ELISAONE™ SMAD1 (p-Ser463/465) assay technical specifications

Specificity:

TGR's ELISAONE SMAD1 (p-Ser463/465) assay kits detect endogenous levels of SMAD1 (GenBank Accession NP_001003688) in cellular lysates. Phospho-SMAD1 assay kits only detect SMAD1 when phosphorylated at Ser463/465. Total SMAD1 assay kits detect SMAD1 irrespective of phosphorylation status. Based on sequence similarity, cross reaction to SMAD5 and SMAD8 may occur.

Species cross-reactivity:

Tested: Human, mouse
Expected: Rat

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

Kit contents:

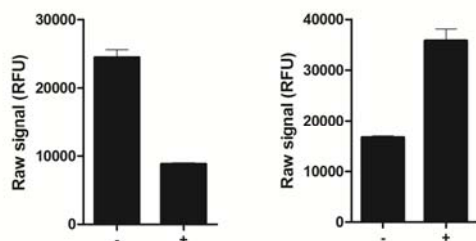
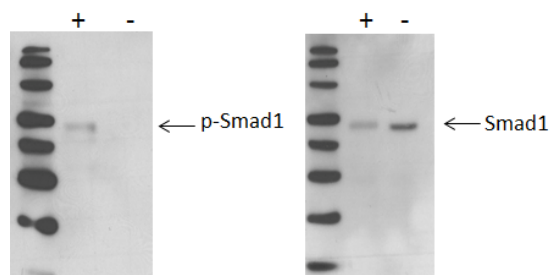
	24-pt Reagent kit	96-well full kit
Capture Antibody Reagent	1 x 0.75 mL	1 x 3.0 mL
Detection Antibody Reagent	1 x 0.75 mL	1 x 3.0 mL
Lysis Buffer (5X)	1 x 2 mL	1 x 8 mL
Enhancer Solution	1 x 0.5 mL	1 x 1 mL
ADHP Dilution Buffer	2 x 1.5 mL	2 x 6 mL
ADHP (100X)	1 x 30 µL	1 x 120 µL
Wash Buffer (10X)	1 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:

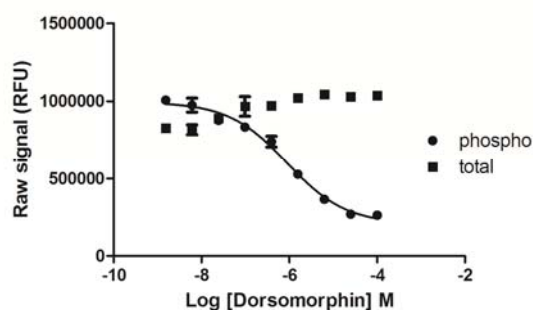
Phospho-SMAD1 and Total-SMAD1 assays are routinely tested against C2C12 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ Smad1 assay performance

Using the Smad1 assay kits or Western blot, Smad1 phosphorylation at Ser463/465 is detected in BMP4-treated C2C12 cells (+), compared with C2C12 cells treated with 50 μ M dorsomorphin (-). Upon phosphorylation, Smad1 levels decrease as Smad1 becomes ubiquitinated.



C2C12 cells were seeded at 5K cells/well in a 96 well tissue culture microplate overnight. The next day cells were serum starved for 60 minutes, then treated with various concentrations of dorsomorphin for 30 mins. Subsequently, the cells stimulated with 50 ng/mL BMP-4 for 30 min. The medium was removed from the wells, and cells were lysed with 120 μ l/well of Lysis Mix, with shaking for 10 min. The lysates were transferred to an ELISAONE assay plate and assayed for either total or phospho-Smad1, 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 1 (S463/465)	24 assays	EKT027
	96 assays	ERS027
Total Smad 1	24 assays	EKT037
	96 assays	ERS037

* 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- κ B Signaling:

NF- κ B p65, I- κ B α , IKK α

Other targets:

p53, CREB1, SMAD1, SMAD3

Wnt Signaling:

β -Catenin, GSK3 β

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.