

ELISAONE™ CREB1 Assays

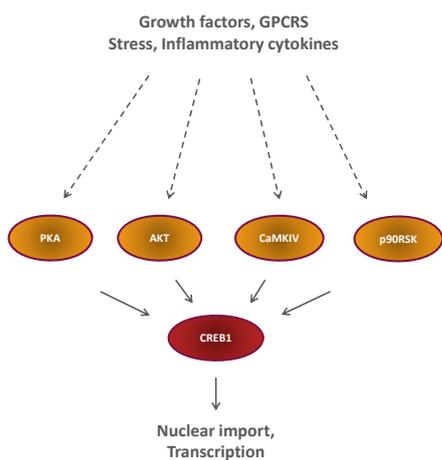
ELISAONE™ assay kits for analysis of cellular CREB1 (p-Ser133) phosphorylation

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-CREB1 and Total CREB1 assays

CREB1 is a member of the ATF/CREB transcription factor family of cyclic-AMP responsive factors that regulate several genes via binding cyclic AMP response elements in the promoter. These genes are important in regulating a broad array of cellular responses. CREB1 is expressed in numerous tissues, and is believed to play a key role in neuronal biology. CREB1 is able to selectively activate various genes through interacting with different dimerization partners.



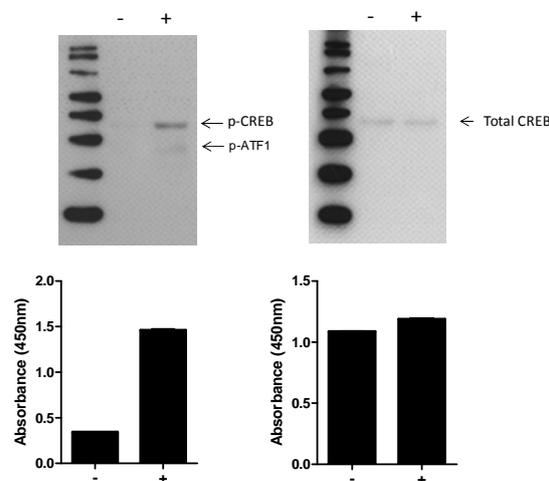
CREB1 activity is regulated by phosphorylation at Ser133, and can be phosphorylated at this site by several different kinases, including those involved in cAMP-mediated, MAPK-mediated, and stress mediated signaling pathways.

ELISAONE™ CREB1 (p-Ser133) assay technical specifications

Specificity:

TGR's ELISAONE CREB1 (p-Ser133) assay kits detect endogenous levels of CREB1 (GenBank Accession NP_004370) in cellular lysates. Phospho-CREB1 assay kits only detect CREB1 when phosphorylated at Ser133. Total CREB1 assay kits detect CREB1 irrespective of phosphorylation status.

As shown, using the CREB1 assay kits or Western blot, CREB1 phosphorylation at Ser133 is detected in EGF-treated A431 cells (+), compared with untreated cells (-), whereas no change in total CREB1 levels was observed.



Species cross-reactivity:

Tested: Human, Mouse, Hamster

Expected: Rat

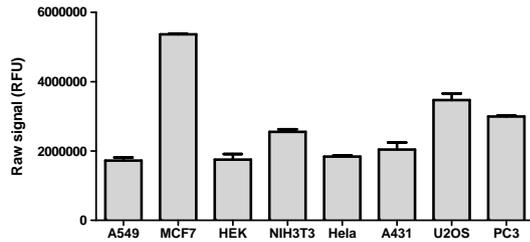
Other species should be tested on a case-by-case basis.

QC:

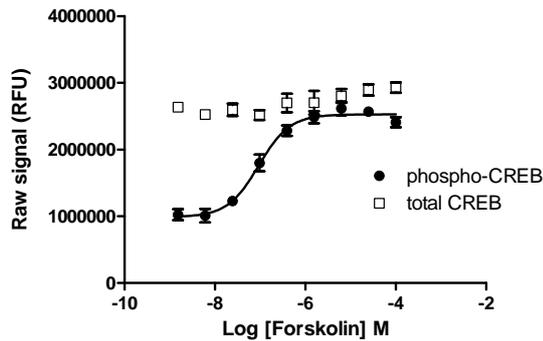
Phospho-CREB1 and total CREB1 assays are routinely tested against EGF-treated A431 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ CREB assay performance

Various cell lines were seeded at 40K/mL in a 96 well plate in medium containing 10% FBS, and cultured overnight. The following day, cells were lysed with 120 µL/well of freshly prepared ELISAONE Lysis Mix with shaking for 10 mins at room temperature. 50 µL of lysate was transferred to replicate wells of an ELISAONE assay plate, and Antibody Mix specific for total CREB (50 µL/well) was added to the lysates. The plates were incubated for 1 hr at room temp, with shaking. Plates were washed and Substrate Mix was added. The plates were covered in foil and incubated for 10 min with shaking. Signal in the wells was determined using a Flexstation (Molecular Devices) plate reader. The results show that CREB is readily detectable in several cell lines.



ELISAONE CREB assay kits are optimized for the rapid detection of CREB in cellular lysates.



A431 cells were seeded at 30K cells/well in medium containing 10% FBS in a 96 well microplate, and cultured overnight. The following day the culture medium was removed, and the cells were serum starved for 60 mins, before treating the cells with varying concentrations of forskolin for 30 min. The medium was removed from the wells, and the cells were lysed with 120 µL/well Lysis Mix, with shaking for 10 min. 50 µL of lysate was transferred to replicate wells of an ELISAONE assay plate, and Antibody Mix specific for either phospho CREB or total CREB (50 µL/well) was added to the lysates. The plates were incubated for 1 hr at room temp, with shaking. Plates were washed and Substrate Mix was added. The plates were covered in foil and incubated for 10 min with shaking. Signal in the wells was determined using a Flexstation (Molecular Devices) plate reader.

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-CREB1 (p-Ser133)	24 assays	ERS024
	96 assays	EKT024
Total CREB1	24 assays	ERS034
	96 assays	EKT034

* ELISAONE assay microplates are not supplied with 24pt kits, and must be purchase 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 (p-Thr308), AKT 1/2/3 (p-Ser473), p70S6K, ribosomal protein S6, GSK3β, BAD

STAT Signaling:

STAT1, STAT3, STAT5

NF-κB Signaling:

NF-κB p65, I-κBα, IKKα

p53 Signaling:

p53

Wnt Signaling:

β-Catenin, GSK3β

TGFβ Signaling:

SMAD1, SMAD3

Protein normalization:

GAPDH

Also available separately:

Product	Pack Size	Catalog
ELISAONE™ 96-well regular microplates	1 plate	EPF001
	5 plates	EPF002
ELISAONE™ 96-well strip-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.