

ELISAONE™ BAD Assays

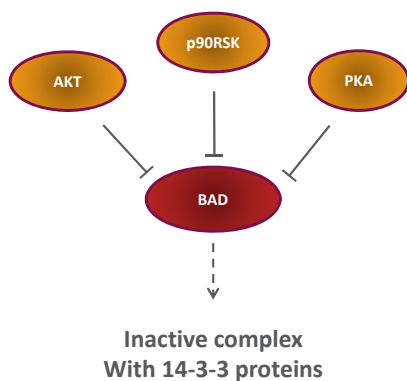
ELISAONE™ assay kits for analysis of cellular BAD (p-Ser112) phosphorylation

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-BAD and Total BAD assays

BAD is a member of the BCL-2 family of proteins, and is involved in the regulation of apoptosis. BAD is a pro-apoptotic protein, and exerts its activity by binding to and inactivating BCL-2 and BCL-xL death repressor activity. BAD binding activity is regulated by phosphorylation. Survival factors (e.g. growth factors, cytokines) inhibit the apoptotic activity of BAD by activating intracellular signaling pathways, including AKT, p90RSK and PKA, which lead to the phosphorylation of BAD at Ser112 and Ser136.



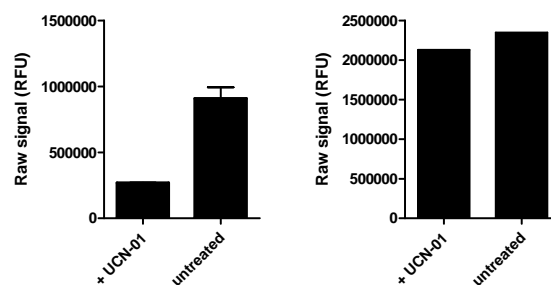
Phosphorylation at these sites promotes binding of BAD to 14-3-3 proteins, thereby preventing BAD binding to BCL-2 or BCL-xL.

ELISAONE™ BAD (p-Ser112) assay technical specifications

Specificity:

TGR's ELISAONE BAD (p-Ser112) assay kits detect endogenous levels of BAD (GenBank Accession NP_004313) in cellular lysates. Phospho-BAD assay kits only detect BAD when phosphorylated at Ser112. Total BAD assay kits detect BAD irrespective of phosphorylation status.

As shown, using the BAD assay kits, BAD phosphorylation at Ser112 is detected in serum-treated PC3 cells (+), compared with UCN-01 treated cells (-). In contrast, no change in total BAD levels was observed.



Species cross-reactivity:

Tested: Human, Mouse

Expected: Rat

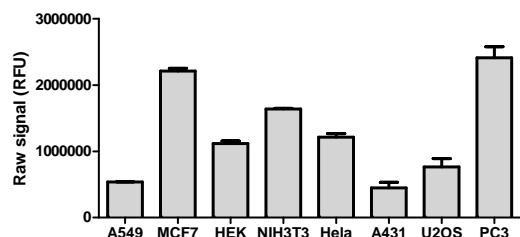
Other species should be tested on a case-by-case basis.

QC:

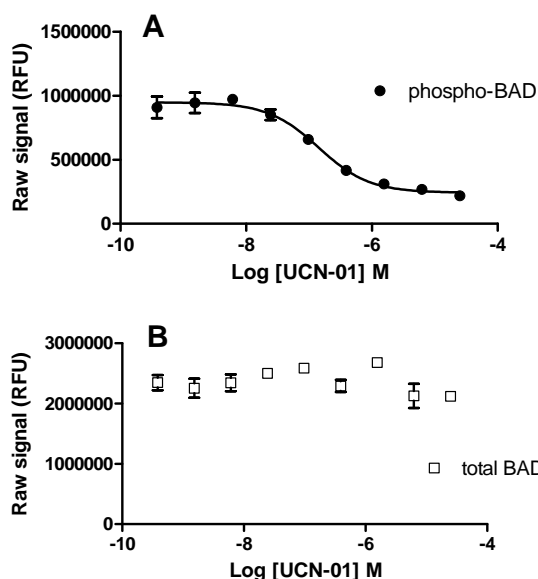
Phospho-BAD and total BAD assays are routinely tested against serum-treated PC3 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ BAD 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 μ L/well of freshly prepared ELISAONE Lysis Mix with shaking for 10 mins at room temperature. 50 μ L of lysate was transferred to replicate wells of an ELISAONE assay plate, and Antibody Mix specific for total BAD (50 μ L/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 BAD is detectable in several cell lines.



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



PC3 cells were seeded at 40K 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 treated with various concentrations of UCN-01 diluted in serum-free medium for 60 mins. The medium was removed from the wells, and the cells were lysed with 120 μ L/well Lysis Mix, with shaking for 10 min. 50 μ L of lysate was transferred to replicate wells of an ELISAONE assay plate, and 50 μ L/well Antibody Mix specific for either (A) phospho BAD or (B) total BAD 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-BAD (S112)	24 assays	ERS041
	96 assays	EKT041
Total BAD	24 assays	ERS051
	96 assays	EKT051

* 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, GSK3 β , BAD

STAT Signaling:

STAT1, STAT3, STAT5

NF- κ B Signaling:

NF- κ B p65, I- κ B α , IKK α

p53 Signaling:

p53

Wnt Signaling:

β -Catenin, GSK3 β

TGF β 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.