

TECHNICAL DATASHEET ELISA – Akt 1/2/3 (p-Thr308)

ELISAONE™ AKT 1/2/3 (p-Thr308) Assays

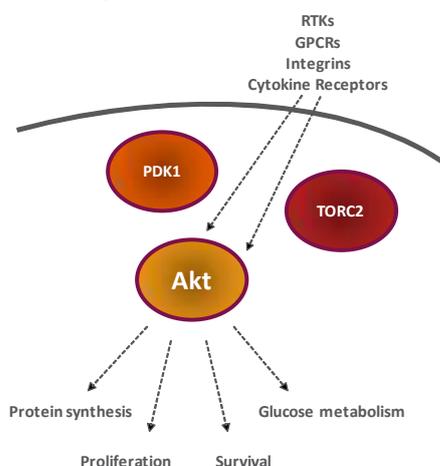
ELISAONE™ assay kits for analysis of cellular AKT 1/2/3 phosphorylation

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-AKT and Total AKT assays

AKT isoforms belong to a conserved family of serine/threonine protein kinases. AKT plays a central role in cell survival and inhibiting apoptosis, by phosphorylating and inactivating several targets. AKT is activated by insulin and various other agonists, which promote transport to the plasma membrane, where the activation loop of AKT is phosphorylated at Thr308 by PDK1. The carboxy terminus is phosphorylated at Ser473 by mammalian target of rapamycin complex 2 (TORC2).



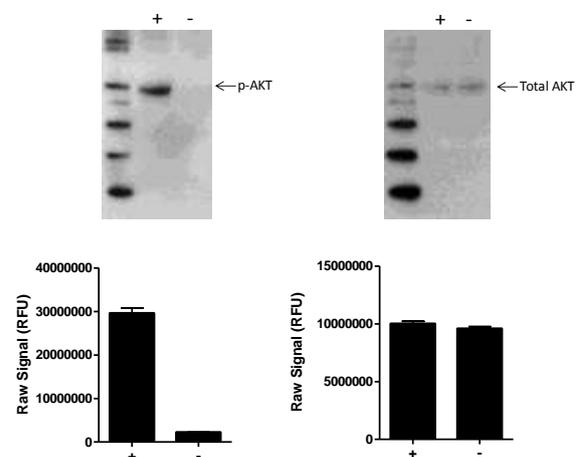
AKT substrates include Bad, forkhead transcription factors, and caspase-9. Another vital AKT function is the regulation of glycogen synthesis through phosphorylation of GSK-3. AKT phosphorylation also prevents GSK-3 β mediated phosphorylation and degradation of cyclin D1, and negatively regulates the cyclin dependent kinase inhibitors p27 Kip and p21 Waf1/CIP1. AKT also plays a critical role in the regulation of TORC1 and tuberlin (TSC2).

ELISAONE™ AKT (p-Thr308) assay technical specifications

Specificity:

TGR's ELISAONE AKT 1/2/3 (p-Thr308) assay kits detect endogenous levels of AKT 1/2/3 (GenBank Accessions NP_001014431 [AKT1], NP_001617 [AKT2], and NP_859029 [AKT3]) in cellular lysates. Phospho-AKT 1/2/3 (p-Thr308) assay kits only detect AKT when phosphorylated at Thr308. Total AKT 1/2/3 assay kits detect AKT 1/2/3 irrespective of phosphorylation status.

As shown below, using the AKT 1/2/3 (p-Thr308) assay kits or Western blot, AKT 1/2/3 phosphorylation at Thr308 is detected in serum-treated HEK293 cells (+), compared with cells treated with 2 μ M wortmannin (-), whereas no change in total AKT 1/2/3 levels was observed.



Species cross-reactivity:

Tested: Human, mouse, hamster

Expected: Rat

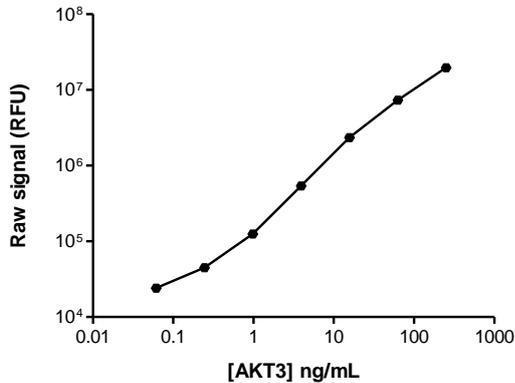
Other species should be tested on a case-by-case basis.

QC:

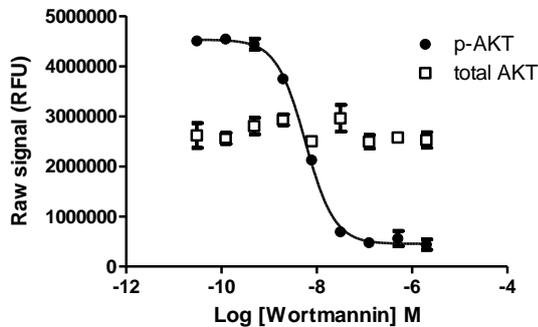
Phospho-AKT and total-AKT assays are routinely tested against HEK293 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ AKT assay performance

Using the ELISAONE phospho-AKT (Thr308) assay, the sensitivity of detection of recombinant human AKT3 was examined. After 60 mins, phospho-AKT concentrations of approximately 200 pg/mL were readily detected. Longer incubation times had little effect on overall limit of detection that was observed. Signal in the wells was determined using a Flexstation (Molecular Devices) plate reader.



ELISAONE AKT assay kits are optimized for the rapid detection of AKT 1/2/3 in cellular lysates.



MCF7 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 wortmannin diluted in serum-free medium for 60 mins, before treating the cells with 2.5 µg/mL insulin 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 AKT or total AKT (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-AKT 1/2/3 (p-Thr308)	24 assays	ERS022
	96 assays	EKT022
Total AKT 1/2/3	24 assays	ERS012
	96 assays	EKT012

* 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.