

ELISAONE™ AKT 1/2/3 Assays

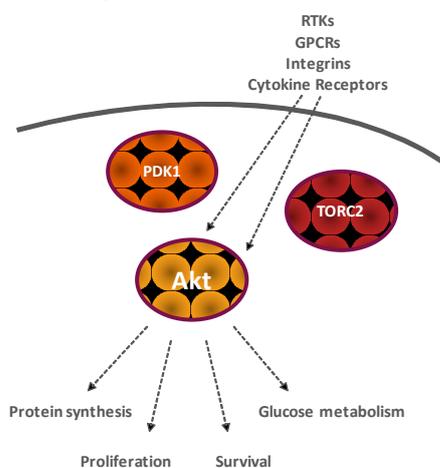
ELISAONE™ assay kits for analysis of cellular AKT 1/2/3 phosphorylation

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).



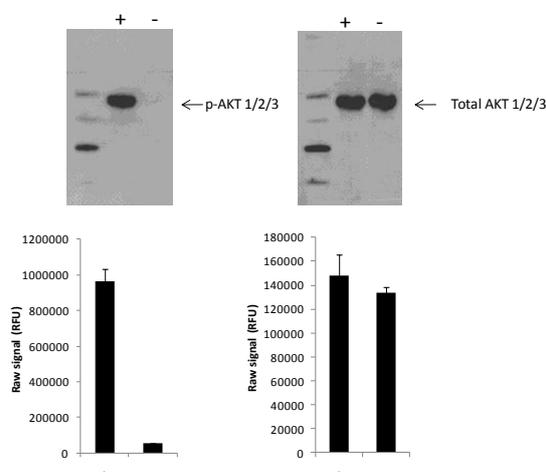
AKT substrates include Bad, forkhead transcription factors, and caspase-9. Another vital AKT function is the regulation of glycogen synthesis through phosphorylation of GSK-3. AKT phosphorylation also prevents GSK-3 β mediated phosphorylation and degradation of cyclin D1, and negatively regulates the 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 assay technical specifications

Specificity:

TGR's ELISAONE AKT 1/2/3 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 assay kits only detect AKT when phosphorylated at Ser473. 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 assay kits or Western blot, a significant increase in AKT 1/2/3 phosphorylation at Ser473 is detected in MCF7 cells treated with insulin for 15 minutes (+), compared with untreated cells (-), whereas no change in total AKT 1/2/3 levels was observed.



Species cross-reactivity:

Tested: Human, mouse, hamster

Expected: Rat

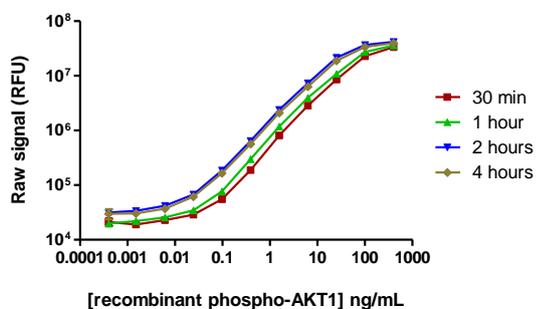
Other species should be tested on a case-by-case basis.

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.

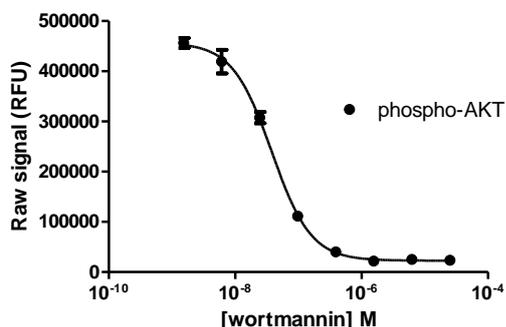
ELISAONE™ AKT assay performance

Using the ELISAONE phospho-AKT assay, the length of assay incubation time that was required to detect recombinant phospho-AKT was examined. After 30 mins, phospho-AKT concentrations of approximately 25 pg/mL were readily detected, while after 1 hour incubation, the limit of detection was less than 10 pg/mL. Longer incubation times had little effect on overall limit of detection that was observed.



	30 min	1 hr	2 hr	4 hr
Limit of Detecton	25 pg/mL	10 pg/mL	10 pg/mL	10 pg/mL

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



Jurkat cells were harvested by centrifugation, resuspended in HBSS, and seeded at 10K/well (20 μ L/well) in a rinsed ELISAONE assay plate. The cells were treated with various concentrations of wortmannin (10 μ L/well) for 1 h at 37°C, then treated with 5% FCS (10 μ L/well) for 15 min. The cells were lysed with 10 μ L/well Lysis Mix Concentrate, with shaking for 10 min. Antibody Mix specific for phospho AKT (50 μ L/well) was added to the lysate, and 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 an Envision (PerkinElmer) 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-Ser473)	24 assays	ERS002
	96 assays	EKT002
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

AKT Signaling:

AKT 1/2/3, p70S6K

STAT Signaling:

STAT3, STAT5

NF- κ B Signaling:

NF- κ B p65, I- κ B α

p53 Signaling:

p53 (p-Ser15)

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

More targets are being developed, including targets for interrogating apoptosis, WNT-signaling, and TGF-beta signaling. Check our website (www.tgrbio.com) for the latest information on target availability.