

ELISAONE™ Ribosomal Protein S6 Assays

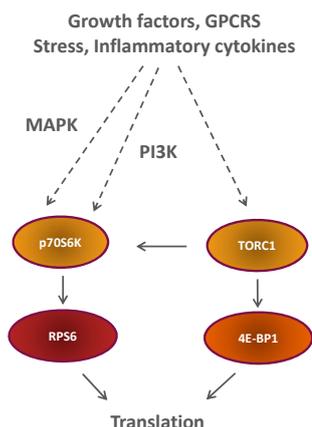
ELISAONE™ assay kits for analysis of cellular RPS6 (p-Ser240/244) phosphorylation

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-RPS6 and Total RPS6 assays

Ribosomes are structures, consisting of a 40S subunit and a 60S subunit, which catalyze protein synthesis. The 40S subunit binds to the mRNA, while the 60S subunit binds to tRNA and the amino acids to be incorporated into the polypeptide being synthesized. RPS6 (Ribosomal Protein S6) is a component of the 40S subunit. RPS6 is phosphorylated at multiple sites in its C-terminal region, which is correlated with an increase in translation of mRNA transcripts.



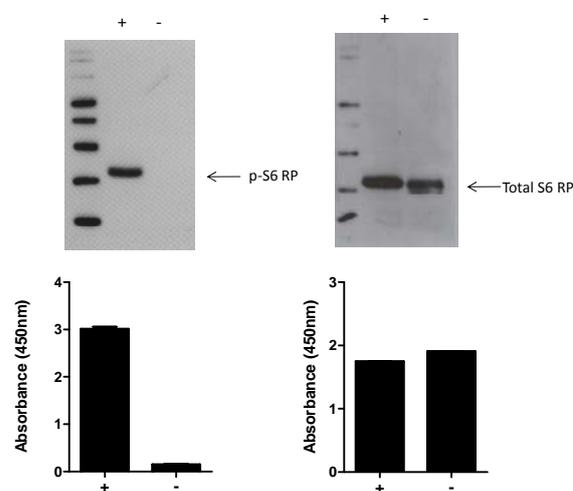
Phosphorylation is induced by many stimuli, including growth factors, and mitogens. RPS6 is thought contribute to the regulation of cell growth and proliferation through the selective translation of classes of mRNA involved in cell cycle progression and protein synthesis.

ELISAONE™ RPS6 (p-Ser240/244) assay technical specifications

Specificity:

TGR's ELISAONE RPS6 (p-Ser240/244) assay kits detect endogenous levels of RPS6 (GenBank Accession NP_001001) in cellular lysates. Phospho-RPS6 assay kits only detect RPS6 when phosphorylated at Ser240/244. Total RPS6 assay kits detect RPS6 irrespective of phosphorylation status.

As shown, using the RPS6 assay kits or Western blot, RPS6 phosphorylation at Ser240/244 is detected in insulin-treated MCF7 cells (+), compared with MCF7 cells treated with a combination of rapamycin, UCN-01 and U0126 (-), whereas no change in total RPS6 levels was observed.



Species cross-reactivity:

Tested: Human, Mouse

Expected: Rat

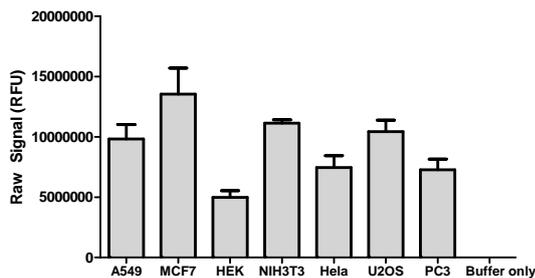
Other species should be tested on a case-by-case basis.

QC:

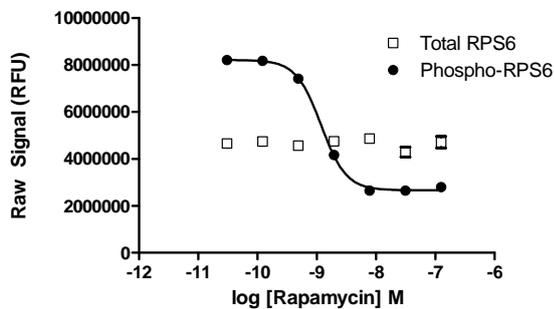
Phospho-RPS6 and total RPS6 assays are routinely tested against inhibitor-treated MCF7 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ RPS6 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 RPS6 (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 RPS6 is readily detectable in several cell lines.



ELISAONE RPS6 assay kits are optimized for the rapid detection of RPS6 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 rapamycin diluted in serum-free medium for 60 mins. 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 RPS6 or total RPS6 (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-RPS6 (p-Ser240/244)	24 assays	ERS042
	96 assays	EKT042
Total RPS6	24 assays	ERS052
	96 assays	EKT052

* 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.