

## ELISAONE™ p70S6K Assays

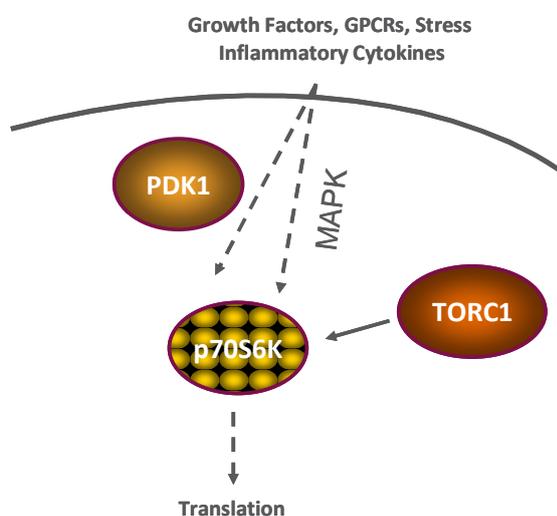
### ELISAONE™ assay kits for analysis of cellular p70S6K phosphorylation

#### 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-p70S6K and Total p70S6K assays

p70S6K is a member of the ribosomal S6 kinase family of Ser/Thr kinases. p70S6K activity is controlled by multiple phosphorylation events, including phosphorylation of Thr389 in the linker domain, which is required for full activation. Activated p70S6K phosphorylates several residues on the S6 ribosomal protein, which ultimately leads to an increase in protein synthesis.



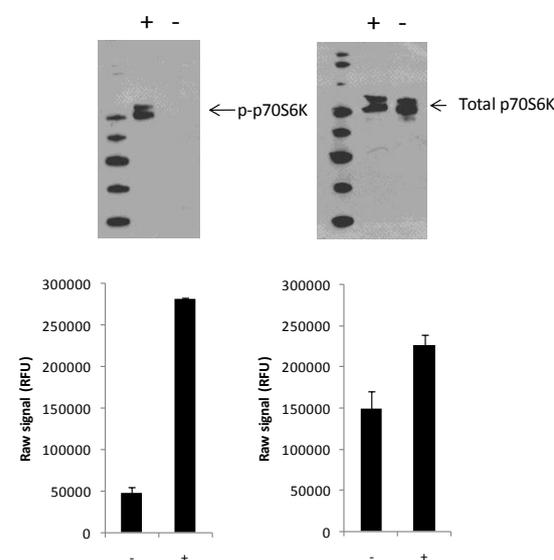
p70S6K is regulated through multiple pathways, including the MAPK pathway, the phosphoinositide-3 kinase (PI-3K) pathway, and the mTOR pathway.

#### ELISAONE™ ERK assay technical specifications

##### Specificity:

TGR's ELISAONE p70S6K assay kits detect endogenous levels of p70S6K (GenBank Accession NP\_003152) in cellular lysates. Phospho-p70S6K assay kits detect p70S6K only when phosphorylated at Thr389. Total p70S6K assay kits detect p70S6K irrespective of phosphorylation status.

Using analysis by either Western blot or ELISAONE p70S6K assay kits, a significant reduction of p70S6K phosphorylation at Thr389 is detected in MCF7 cells treated with a combination of UCN-01, wortmannin and rapamycin for 3 hours (-), compared with untreated cells (+), while only a small change in total p70S6K levels was observed.



##### Species cross-reactivity:

Tested: Human, mouse

Expected: Rat

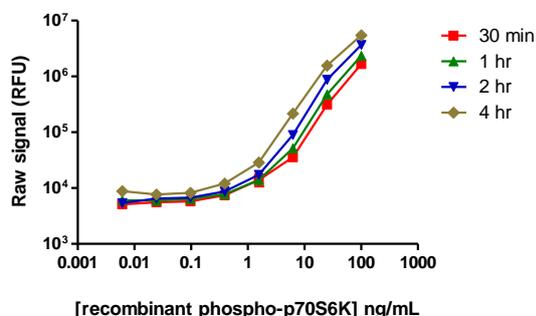
Other species should be tested on a case-by-case basis.

##### QC:

Phospho-p70S6K and Total-p70S6K assays are routinely tested against MCF7 cellular lysates. See certificate of analysis for Lot-specific information. Available at [www.tgrbio.com](http://www.tgrbio.com).

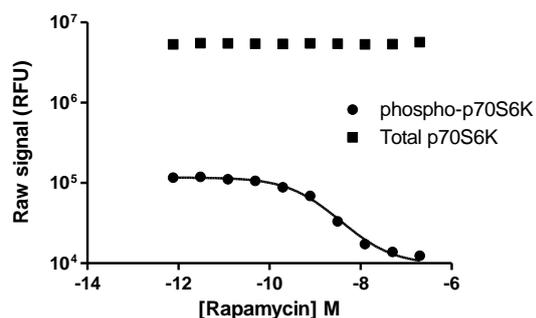
### ELISAONE™ p70S6K assay performance

Using the ELISAONE phospho-p70S6K assay, the length of assay incubation time that was required to detect recombinant phospho-p70S6K was examined. After 30 mins, phospho-p70S6K concentrations of approximately 0.5 ng/mL were readily detected. Longer incubation times had little effect on overall limit of detection that was observed.



	30 min	1 hr	2 hr	4 hr
Limit of Detection	0.5 ng/mL	0.5 ng/mL	0.5 ng/mL	0.5 ng/mL

ELISAONE p70S6K assays are optimized for the rapid detection of p70S6K in cellular lysates.



**Day 1:** PC-3 Cells were seeded at 40K cells/well in a 96 well tissue culture plate, in medium containing 10% FBS.

**Day 2:** The media was removed from the wells, and the cells were treated with various concentrations of rapamycin in serum-free media for 2 hrs, before the cells were further treated with 10 µg/mL insulin for 30 min. The media was removed from the wells, and the cells were lysed with 125 µL/well freshly prepared ELISAONE Lysis Mix, with shaking for 10 min at room temp. Lysates (50 µL) were transferred to replicate wells of an ELISAONE assay plate and analyzed for either phospho-p70S6K, or Total p70S6K, using the standard ELISAONE protocol. Briefly, Antibody Mix specific for either phospho-p70S6K, or Total p70S6K was added to the lysates, and the plate was incubated for 1 hr at room temp, with shaking. The plate was 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 plate reader (Molecular Devices).

### 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-p70S6K (p-Thr389)	24 assays	ERS010
	96 assays	EKT010
Total p70S6K	24 assays	ERS020
	96 assays	EKT020

\* 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-κB Signaling:**

NF-κB p65, I-κBα

**p53 Signaling:**

p53

**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](http://www.tgrbio.com)) for the latest information on target availability.