

ELISAONE™ p38 MAPK Assays

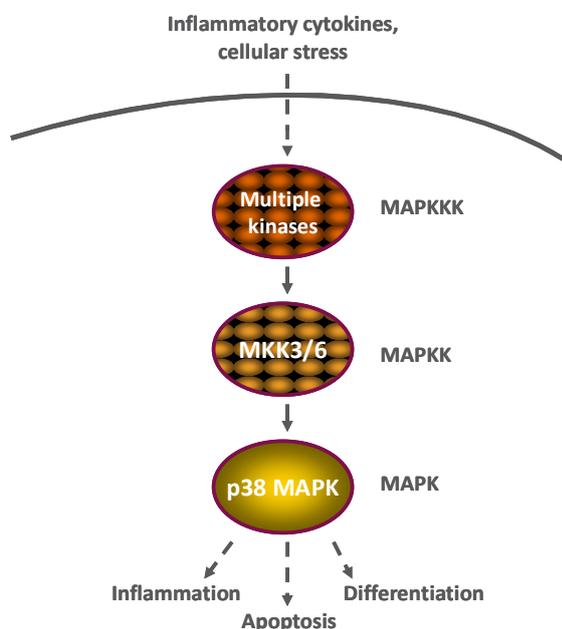
ELISAONE™ assay kits for analysis of cellular p38 MAPK phosphorylation

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-p38 MAPK and Total p38 MAPK assays

p38 MAPK is a member of the MAP kinase family, which are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. In particular, p38 MAPK is activated by phosphorylation at Thr180 and Tyr182, which is mediated by a variety of cellular stresses including inflammatory cytokines, UV light, osmotic shock and lipopolysaccharides.



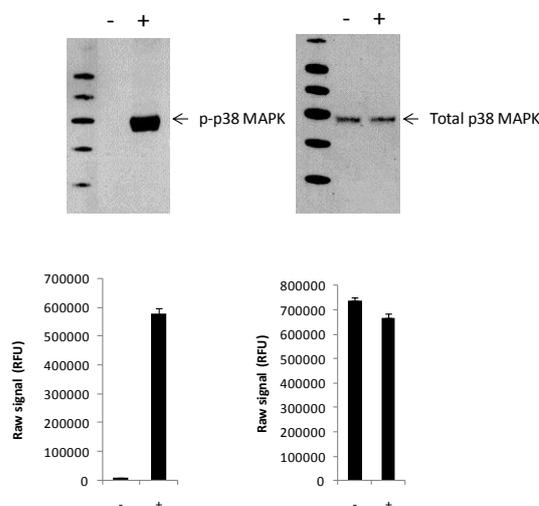
Activated p38 MAP kinase has been shown to phosphorylate and activate several downstream kinases and transcription factors implicated in regulating stress related transcription and the control of cell cycle.

ELISAONE™ p38 MAPK assay technical specifications

Specificity:

TGR's ELISAONE p38 MAPK assay kits detect endogenous levels of p38 MAPK (GenBank Accession NP_001306) in cellular lysates. Phospho-p38 MAPK assay kits detect p38 MAPK only when phosphorylated at Thr180/Tyr182. Total p38 MAPK assay kits detect p38 MAPK irrespective of phosphorylation status.

As shown below, analysis by either Western blot, or ELISAONE p38 MAPK assay kits, revealed a significant stimulation of p38 MAPK phosphorylation at Thr180/Tyr182 is detected in HEK293 cells treated with anisomycin for 30 minutes compared with untreated cells, (B) while no change in total p38 MAPK levels is observed.



Species cross-reactivity:

Tested: Human, mouse

Expected: Rat

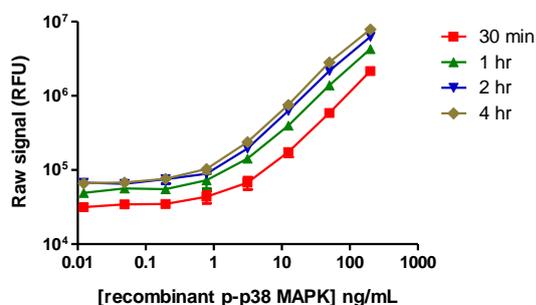
Other species should be tested on a case-by-case basis.

QC:

Phospho-p38 MAPK and Total p38 MAPK assays are routinely tested against HEK293 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

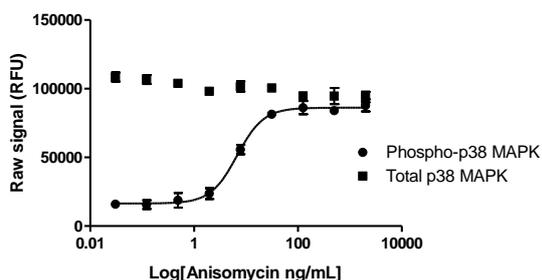
ELISAONE™ p38 MAPK assay performance

Using the ELISAONE phospho-p38 MAPK assay, the length of assay incubation time that was required to detect recombinant phospho-p38 MAPK was examined. After 30 mins, phospho-p38 MAPK concentrations of approximately 1 ng/mL were readily detected, while after 1 hour incubation, the limit of detection remained approximately 1 ng/mL, but with a much improved assay window. Incubation times longer than one hour showed a slight improvement on assay performance.



	30 min	1 hr	2 hr	4 hr
Limit of Detection	1 ng/mL	1 ng/mL	0.2 ng/mL	0.2 ng/mL

ELISAONE p38 MAPK assays are optimized for the rapid detection of p38 MAPK in cellular lysates.



Day 1: HeLa cells were harvested from 80% confluent flasks, and seeded at 25K cells/well in a 96 well tissue culture plate in medium containing 10% FBS and incubated overnight.

Day 2: The medium was removed, and replaced with serum free medium and incubated overnight.

Day 3: The cell were treated with various concentration of anisomycin for 15 minutes. The media was removed, and the cells were lysed with 125 μ L/well freshly prepared Lysis Mix, with shaking for 10 min. Lysates (50 μ L) were transferred to replicate wells of an ELISAONE assay plate and analyzed for either phospho or total p38 MAPK using the standard ELISAONE protocol. Briefly, Antibody Mix specific for either phospho-p38 MAPK, or Total p38 MAPK (50 μ L/well), was added to the lysate, and the plate was incubated for 1 hr at room temp, with shaking. The plate was washed and Substrate Mix was added to the wells. The plate was 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-p38 MAPK (p-Thr180/Tyr182)	24 assays	ERS008
	96 assays	EKT008
Total p38 MAPK	24 assays	ERS018
	96 assays	EKT018

* ELISAONE assay microplates are not supplied with 24pt kits, but can 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

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.