

## ELISAONE™ JNK 1/2/3 Assays

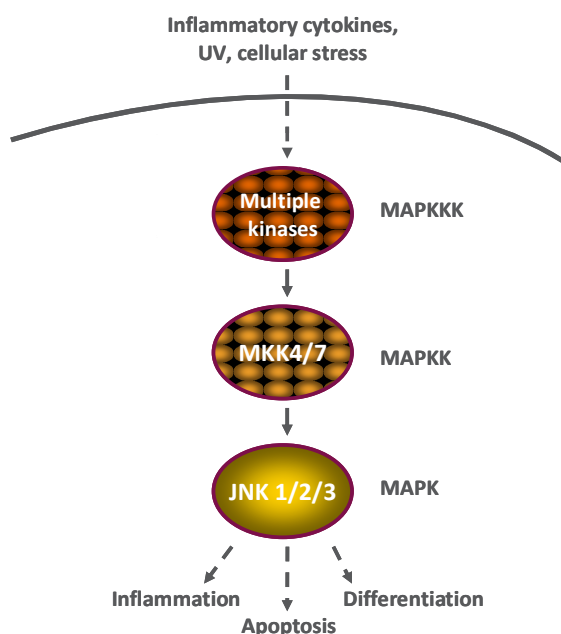
ELISAONE™ assay kits for analysis of cellular JNK 1/2/3 phosphorylation

### 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-JNK and Total JNK assays

c-Jun N-terminal kinases (JNKs) are mitogen-activated protein kinases which are activated in response to stress stimuli, including inflammatory cytokines, ultraviolet irradiation, heat shock, and osmotic shock. JNK proteins are encoded by three genes, JNK1, JNK2 and JNK3, which undergo alternate splicing to generate several isoforms for each gene.



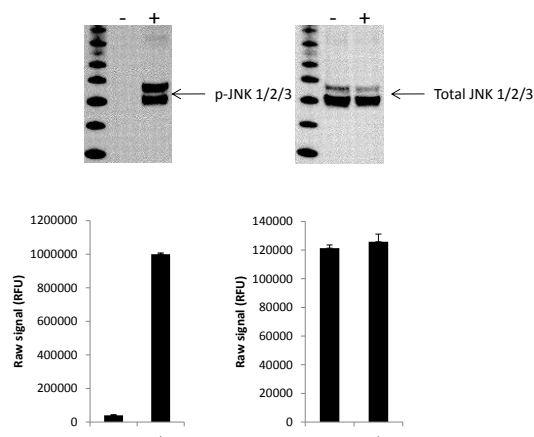
JNK proteins are activated by phosphorylation, which is mediated through a classical MAPKKK/MAPKK/MAPK cascade, which in turn can be initiated through several mechanisms, generally in response to cellular stress signals. Activated JNKs can phosphorylate several proteins, including the transcription factor c-JUN.

### ELISAONE™ JNK assay technical specifications

#### Specificity:

TGR's ELISAONE JNK 1/2/3 assay kits detect endogenous levels of JNK 1/2/3 (GenBank Accessions NP\_620637 [JNK1], NP\_620707 [JNK2] and NP\_620446 [JNK3]) in cellular lysates. Phospho-JNK 1/2/3 assay kits detect JNK only when phosphorylated at Thr183/Tyr185. Total JNK 1/2/3 assay kits detect JNK irrespective of phosphorylation status.

As shown below, using the JNK 1/2/3 assay kits, (A) a significant stimulation of JNK 1/2/3 phosphorylation at Thr183/Tyr185 is detected in HEK293 cells treated with anisomycin for 30 minutes compared with untreated cells, (B) while no change in total JNK levels is observed.



#### Species cross-reactivity:

Tested: Human, mouse  
Expected: Rat

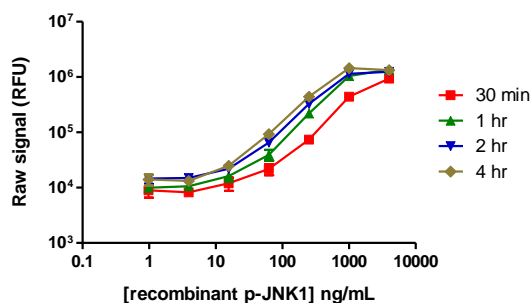
Other species should be tested on a case-by-case basis.

#### QC:

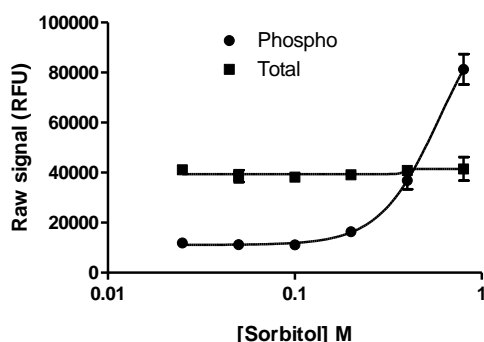
Phospho-JNK and Total-JNK assays are routinely tested against HEK293 cellular lysates. See certificate of analysis for Lot-specific information. Available at [www.tgrbio.com](http://www.tgrbio.com).

## ELISAONE™ JNK assay performance

Using the ELISAONE phospho-JNK assay, the length of assay incubation time that was required to detect a recombinant phospho-JNK1-GST fusion protein was examined. After 30 mins, phospho-JNK concentrations of approximately 16 ng/mL were readily detected, while after 1 hour incubation, the limit of detection was approximately the same, but with a much improved assay window. Incubation times longer than one hour had little effect on overall assay performance.



ELISAONE JNK assays are optimized for the rapid detection of JNK 1/2/3 in cellular lysates.



U937 were harvested from flasks of cells in logarithmic growth phase, and resuspended in HBSS containing 5% FBS. The cells were seeded at 50K cells/well in a 96 well tissue culture plate and incubated at 37°C for 1 hour. The cells were treated with various concentrations of sorbitol, diluted in HBSS/0.1% BSA, for 30 minutes, then the cells were lysed with Lysis Mix Concentrate, with shaking for 10 minutes. Lysates (50 µL) were transferred to replicate wells of an ELISAONE assay plate and analyzed for either phospho or total JNK 1/2/3 using the standard ELISAONE protocol. Briefly, Antibody Mix specific for either phospho-JNK 1/2/3, or Total JNK 1/2/3 (50 µL/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-JNK 1/2/3 (p-Thr183/Tyr185)	24 assays	ERS007
	96 assays	EKT007
Total JNK 1/2/3	24 assays	ERS017
	96 assays	EKT017

\* 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-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.