

ELISAONE™ ERK 1/2 Assays

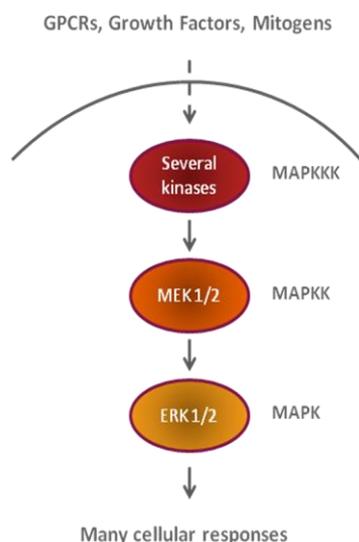
ELISAONE™ assay kits for analysis of cellular ERK 1/2 phosphorylation

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-ERK and Total ERK assays

ERK1 and ERK2, belong to a conserved family of serine/threonine protein kinases. ERK 1/2 form part of a classical MAPK signaling cascade, consisting of a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK), and a MAP kinase (MAPK).



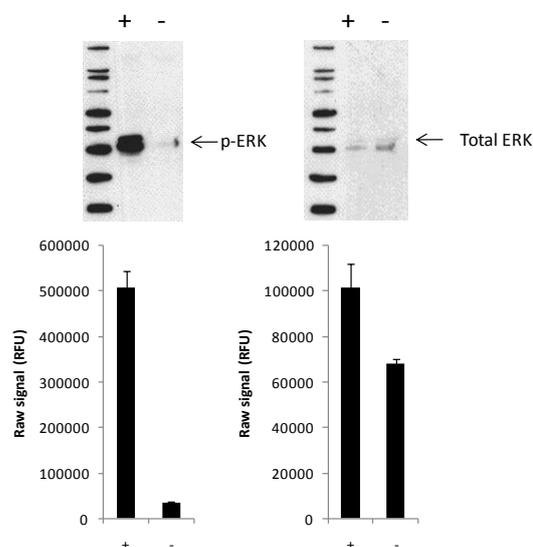
ERK 1/2 are involved in cellular signaling events associated with a range of stimuli, including GPCRs, growth factors, and cytokines. The kinase activity of ERK proteins is regulated by dual phosphorylation at Thr202/Tyr204 in ERK1, and Threonine 185/Tyrosine 187 in ERK2. MEK1 and MEK2 are the primary upstream kinases responsible for ERK 1/2 phosphorylation in this pathway. Several targets of ERK 1/2 have been identified, including downstream kinases and transcription factors.

ELISAONE™ ERK assay technical specifications

Specificity:

TGR's ELISAONE ERK 1/2 assay kits detect endogenous levels of ERK 1/2 (GenBank Accessions NP 002737.2 [ERK1], NP 620407 [ERK2]) in cellular lysates. Phospho-ERK 1/2 assay kits detect ERK only when phosphorylated at Thr202/Tyr204. Total ERK 1/2 assay kits detect ERK irrespective of phosphorylation status.

As shown below, using (A) the ERK 1/2 assay kits, a significant reduction of ERK 1/2 phosphorylation at Thr202/Tyr204 is detected in A431 cells treated with AG1478 for 3 hours compared with untreated cells, while no change in total ERK levels (B) is observed.



Species cross-reactivity:

Tested: Human, mouse, hamster

Expected: Rat

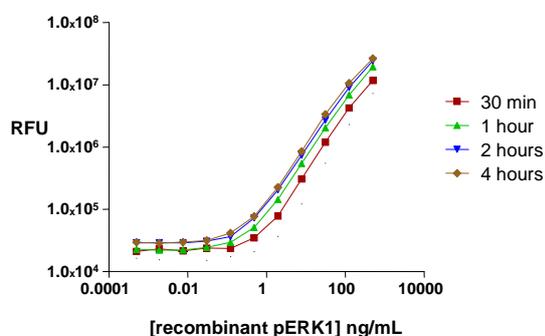
Other species should be tested on a case-by-case basis.

QC:

Phospho-ERK and Total-ERK assays are routinely tested against A431 cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

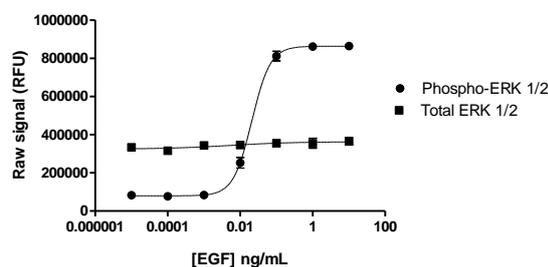
ELISAONE™ ERK assay performance

Using the ELISAONE phospho-ERK assay, the length of assay incubation time that was required to detect recombinant phospho-ERK was examined. After 30 mins, phospho-ERK concentrations of approximately 0.5 ng/mL were readily detected, while after 1 hour incubation, the limit of detection was approximately 0.1 ng/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 Detection	0.5 ng/mL	0.1 ng/mL	0.1 ng/mL	0.1 ng/mL

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



Day 1: HEK293 cells were seeded at 25,000 cells/well in a 96 well tissue-culture microplate, in medium containing 10% FBS.

Day 2: the cells were serum-starved overnight.

Day 3: the cells were treated with various concentrations of EGF for 15 min. The media was removed from the wells, and the cells were lysed with 130 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-ERK, or Total ERK using the standard ELISAONE protocol. Briefly, Antibody Mix (50 µL/well) specific for either phospho-ERK 1/2, or Total ERK 1/2, 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-ERK 1/2 (p-Thr202/Tyr204)	24 assays	ERS001
	96 assays	EKT001
Total ERK 1/2	24 assays	ERS011
	96 assays	EKT011

* ELISAONE assay microplates are not supplied with 24pt kits, but can be purchased 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) for the latest information on target availability.