

ELISAONE™ NF- κ B p65 Assays

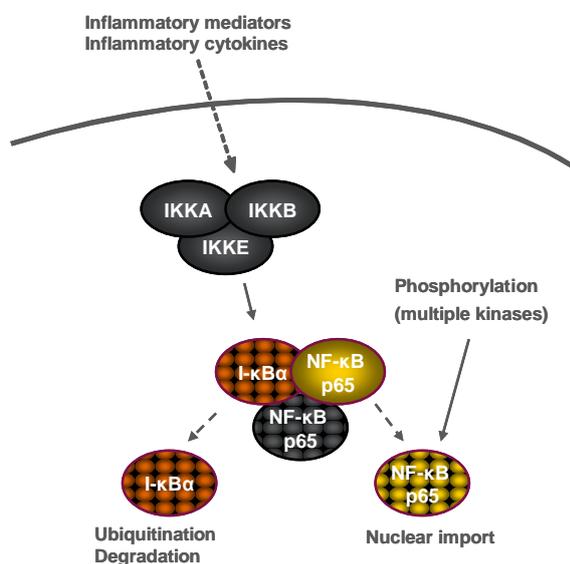
ELISAONE™ assay kits for analysis of cellular NF- κ B p65 phosphorylation

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-NF- κ B p65 and Total NF- κ B p65 assays

The nuclear factor κ B transcription factor family plays a pivotal role in inflammatory and immune responses. There are five family known members, namely p65 (RelA), c-Rel, RelB, NF- κ B1 (p105/p50) and NF- κ B2 (p100/p52). The p50 and p52 products form dimeric complexes with Rel proteins, which bind DNA and regulate transcription. The most abundant form in many cell types is the p65/p50 complex.



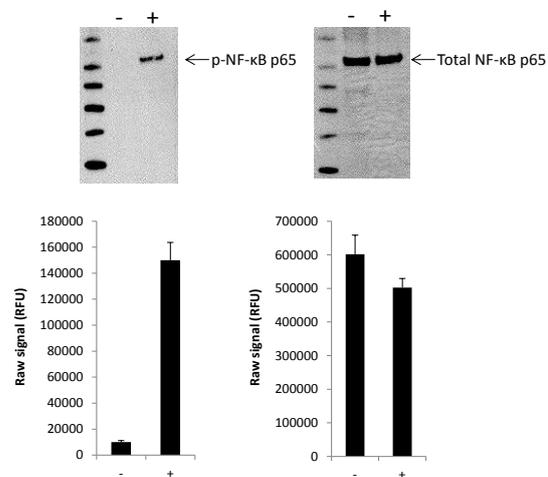
In unstimulated cells, NF- κ B is inactive in the cytoplasm, bound by I- κ B proteins. Activation of the NF- κ B pathway induces phosphorylation of I- κ Bs, targeting them for degradation through the ubiquitin-proteasome pathway. Free NF- κ B can then enter the nucleus, where it regulates expression of its target genes.

ELISAONE™ NF- κ B p65 assay technical specifications

Specificity:

TGR's ELISAONE NF- κ B p65 assay kits detect endogenous levels of NF- κ B p65 (GenBank Accession NP_068810) in cellular lysates. Phospho-NF- κ B p65 assay kits detect NF- κ B p65 only when phosphorylated at Ser536. Total NF- κ B p65 assay kits detect NF- κ B p65 irrespective of phosphorylation status.

As shown below, using the NF- κ B p65 assay kits, (A) a significant stimulation of NF- κ B p65 phosphorylation at Ser536 is detected in HeLa cells treated with TNF α for 10 minutes compared with untreated cells, (B) while no change in total NF- κ B p65 levels is observed.



Species cross-reactivity:

Tested: Human

Expected: Mouse, Rat

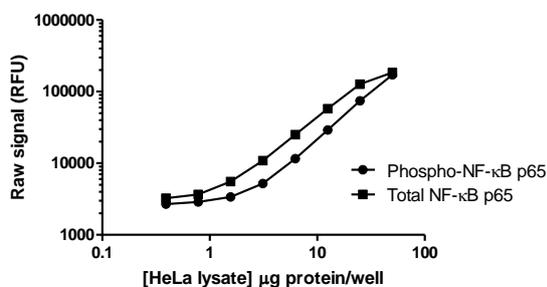
Other species should be tested on a case-by-case basis.

QC:

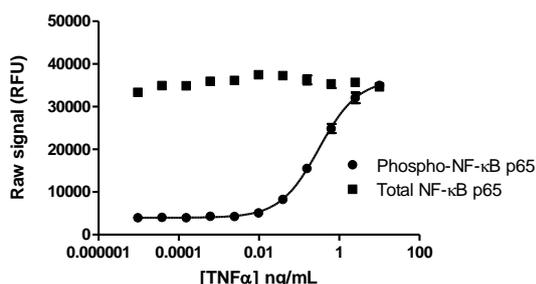
Phospho-NF- κ B p65 and Total-NF- κ B p65 assays are routinely tested against HeLa cellular lysates. See certificate of analysis for Lot-specific information. Available at www.tgrbio.com.

ELISAONE™ NF-κB p65 assay performance

Using the ELISAONE phospho-NF-κB p65 and Total NF-κB p65 assays, the amount of lysate required to detect NF-κB p65 in TNFα-treated HeLa cell lysates was examined. Up to 50 μg of lysate/well was loaded into replicate wells of an ELISAONE microplate, and analysed for either phospho-NF-κB p65 or total NF-κB p65. Both phospho-NF-κB p65 and total NF-κB p65 were readily detected in less than 10 μg of lysate/well.



ELISAONE NF-κB p65 assays are optimized for the rapid detection of NF-κB p65 in cellular lysates.



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

Day 2: The media was removed and cells were stimulated with various concentrations of TNFα for 10 minutes. The media was removed, and the cells were lysed with 125 μL/well freshly prepared Lysis Mix, with shaking for 10 min. Lysates (50 μL) were transferred to replicate wells of an ELISAONE assay plate and analyzed for either phospho or total NF-κB p65 using the standard ELISAONE protocol. Briefly, Antibody Mix specific for either phospho-NF-κB p65, or Total NF-κB p65 (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 Victor3 plate reader (Wallac).

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-NF-κB p65 (p-Ser536)	24 assays	ERS003
	96 assays	EKT003
Total NF-κB p65	24 assays	ERS013
	96 assays	EKT013

* 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) for the latest information on target availability.