Datasheet for ABIN965407

NF-kB p65 ELISA Kit

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Overview

Quantity:	1 kit
Target:	NF-kB p65 (NFkBP65)
Reactivity:	Human
Method Type:	DNA-Binding ELISA
Application:	ELISA

Product Details

Purpose:	NF-kB (p65) Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	A 96 well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the NF-kB response element is immobilized onto the bottom of wells of a 96 well plate (see Figure 1 on page 4). NF-kB contained in a nuclear extract specifically binds to the NF-kB response element. NF-kB (p65) is detected by addition of a specific primary antibody directed against NF-kB (p65). A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. NF-kB (p65) Transcription Factor Assay detects human NF-kB (p65). It will not cross-react with NF-kB (p50).

Target Details

Target:	NF-kB p65 (NFkBP65)

Target Details

Alternative Name:	NF kB (p65) (NFkBP65 Products)
Background:	The NF-kB/Rel family of transcription factors is comprised of several structurally related proteins that form homodimers and heterodimers and include p50/p105, p52/p100, RelA (p65), c-Rel/NF-kB [1]. Members of this family are responsible for regulating over 150 target genes, including the expression of inflammatory cytokines, chemokines, immunoreceptors and cell adhesion molecules. Because of this, NF-kB has often been called a 'central mediator of the human immune response' [2]. Acting as dimers, these transcription factors bind to DNA sequences, collectively called kB, sites thereby regulating expression of target genes. In most cells, Rel/ NF-kB transcription complexes are present in an inactive form in the cytoplasm, bound to an inhibitor I□B. Certain stimuli result in the phosphorylation, ubiquitination and subsequent degradation of IkB proteins thereby enabling translocation of NF-kB into the nucleus [3]. The most common Rel/NF-kB dimmer in mammals contains p50-RelA (p50/p65) heterodimers and is specifically called NF-kB. One of the target genes activated by NF-kB is that encoding IkBα. This feedback mechanism allows newly-synthesized IkBα to enter the nucleus, remove NF-kB from DNA and transport it back to the cytoplasm thereby restoring its inactive state. The importance of Rel/NF-kB transcription factors in human inflammation and certain diseases makes them attractive targets for potential therapeutics [4-6]. Synonyms: NF-kB Transcription Factor Kit, p65 kit, EIA kit, NF-κB (p65) Transcription Factor Assay
Pathways:	NF-kappaB Signaling, RTK Signaling, TCR Signaling, TLR Signaling, Fc-epsilon Receptor Signaling Pathway, Neurotrophin Signaling Pathway, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Hepatitis C, Toll-Like Receptors Cascades, S100 Proteins

Application Details

Application Notes:	NF-kB (p65) Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A 96 well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the NF-kB response element is immobilized onto the bottom of wells of a 96 well plate (see Figure 1 on page 4). NF-kB contained in a nuclear extract specifically binds to the NF-kB response element. NF-kB (p65) is detected by addition of a specific primary antibody directed against NF-kB (p65). A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. NF-kB (p65) Transcription Factor Assay detects human NF-kB (p65). It will not cross-react with NF-kB (p50).

Application Details

Comment:	Detection Kit Type: ELISA Kit
Plate:	Pre-coated
Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C

Publications

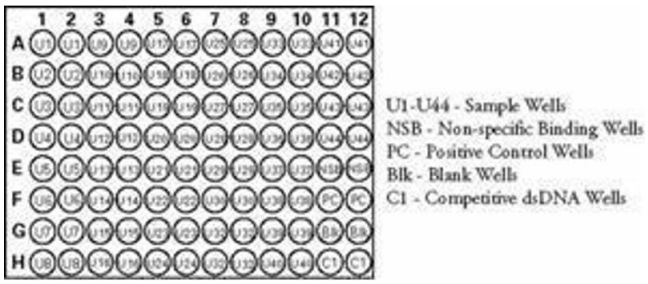
Product cited in:

Gupta, Campbell, Dérijard, Davis: "Transcription factor ATF2 regulation by the JNK signal transduction pathway." in: **Science (New York, N.Y.)**, Vol. 267, Issue 5196, pp. 389-93, (1995) (PubMed).

Livingstone, Patel, Jones: "ATF-2 contains a phosphorylation-dependent transcriptional activation domain." in: **The EMBO journal**, Vol. 14, Issue 8, pp. 1785-97, (1995) (PubMed).

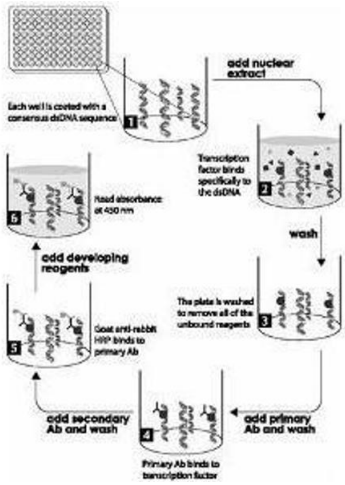
van Dam, Wilhelm, Herr, Steffen, Herrlich, Angel: "ATF-2 is preferentially activated by stress-activated protein kinases to mediate c-jun induction in response to genotoxic agents." in: **The EMBO journal**, Vol. 14, Issue 8, pp. 1798-811, (1995) (PubMed).

Abdel-Hafiz, Heasley, Kyriakis, Avruch, Kroll, Johnson, Hoeffler: "Activating transcription factor-2 DNA-binding activity is stimulated by phosphorylation catalyzed by p42 and p54 microtubule-associated protein kinases." in: **Molecular endocrinology (Baltimore, Md.)**, Vol. 6, Issue 12, pp. 2079-89, (1993) (PubMed).



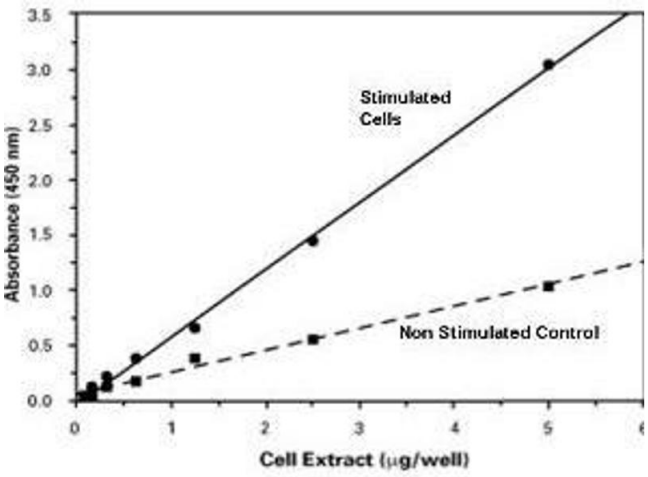
ELISA

Image 1.



ELISA

Image 2. Schematic of the Transcription Factor Binding Assay.



ELISA

Image 3. Transcription factor assay absorbance of cell lysates isolated from stimulated (20 ng/mL TNFa for 30 min.) and non-stimulated HeLa cells demonstrating NF-?B (p50) activity.