

Datasheet for KAA065**NFkB p65 ELISA Kit****Overview**

Description:	NFkB (P65) ELISA Kit - KAA065
Item No.:	KAA065
Size:	1 Kit
Applications:	ELISA, Other
Reactivity:	Human

Product Details

Background:	The NF- κ B/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- κ B [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- κ B 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 κ B, sites thereby regulating expression of target genes. In most cells, Rel/ NF- κ B transcription complexes are present in an inactive form in the cytoplasm, bound to an inhibitor I κ B. Certain stimuli result in the phosphorylation, ubiquitination and subsequent degradation of I κ B proteins thereby enabling translocation of NF- κ B into the nucleus [3]. The most common Rel/NF- κ B dimer in mammals contains p50-RelA (p50/p65) heterodimers and is specifically called NF- κ B. One of the target genes activated by NF- κ B is that encoding I κ B α . This feedback mechanism allows newly-synthesized I κ B α to enter the nucleus, remove NF- κ B from DNA and transport it back to the cytoplasm thereby restoring its inactive state. The importance of Rel/NF- κ B transcription factors in human inflammation and certain diseases makes them attractive targets for potential therapeutics [4-6].
Synonyms:	NF- κ B Transcription Factor Kit, p65 kit, EIA kit, NF- κ B p65 Transcription Factor Assay
Detection Kit Type:	ELISA Kit

Target Details

Reactivity:	Human
Relevant Links:	NF-κB (P65) ELISA Kit Protocol

Application Details

Tested Applications:	ELISA
Suggested Applications:	Other (Based on references)
Application Note:	Rockland's 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. Rockland's NF-kB (p65) Transcription Factor Assay detects human NF-kB (p65). It will not cross-react with NF-kB (p50).
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:100
Other:	Expiration Date: 31 AUG 2022

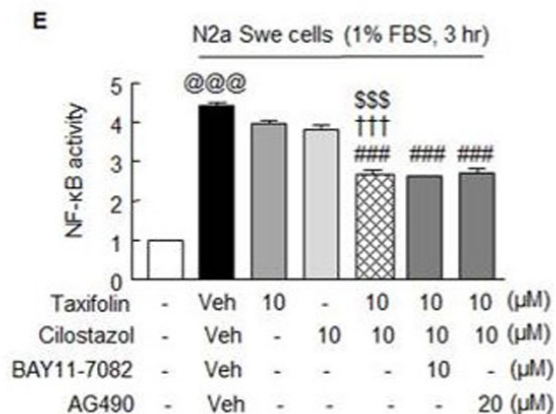
Formulation

Physical State:	n/a
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Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	See kit insert for complete instructions.
Expiration:	See kit insert for complete instructions.

Images

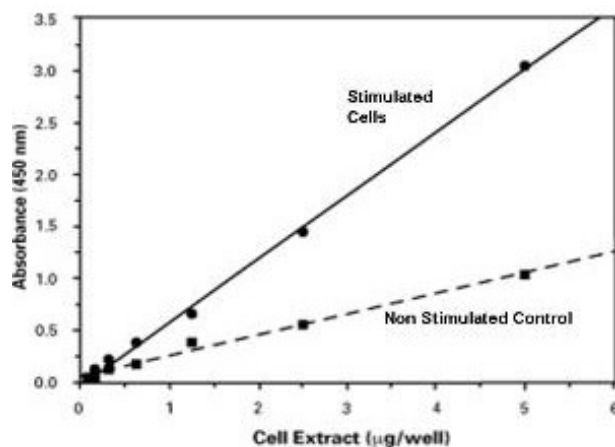
**Figure**

Aβ-induced changes in the expressions of NF-κB p65, and effects of taxifolin and cilostazol in N2a Swe cells. E. Significant decrease in DNA binding activity of NF-κB by co-treatment with 10 μM taxifolin and 10 μM cilostazol as compared with taxifolin or cilostazol monotherapy. NF-κB p65 DNA binding activities were determined using a colorimetric NF-κB p65 transcription factor assay kit (p/n KAA065). Results are the means ± SEMs of 4 experiments. *P < 0.05, **P < 0.01, ***P < 0.001 vs. Vehicle (Veh); \$ \$ \$P < 0.001 vs. Cilostazol alone; ††† P < 0.001 vs. 10 μM Taxifolin alone; ### P < 0.001 vs. Vehicle (Veh); @@@P < 0.001 vs. None.

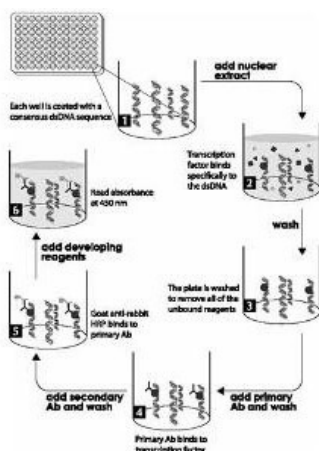
Fig 3. PMID: 27977755.

**Kit Box**

This product is assembled as a kit. See attached protocol or CofA for further details.

**ELISA**

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



ELISA

NF-kB Transcription Factor Binding Assay Schematic

ELISA

NF-kB Sample Plate Format

	1	2	3	4	5	6	7	8	9	10	11	12
A	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
B	U2	U2	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
C	U3	U3	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
D	U4	U4	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
E	U5	U5	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
F	U6	U6	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
G	U7	U7	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1
H	U8	U8	U1	U1	U1	U1	U1	U1	U1	U1	U1	U1

U1-U44 - Sample Wells
 NSB - Non-specific Binding Wells
 PC - Positive Control Wells
 Blk - Blank Wells
 C1 - Competitive dsDNA Wells

References

- Park SY et al. Concurrent Treatment with Taxifolin and Cilostazol on the Lowering of β -Amyloid Accumulation and Neurotoxicity via the Suppression of P-JAK2/P-STAT3/NF- κ B/BACE1 Signaling Pathways. *PLoS One* (2016)

Disclaimer

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