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Detection of total NF-κB using an immunometric transcription factor assay kit based on anti-RelA (p65) antibody.

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Rockland Immunochemicals has developed the first of a series of new antibody based ELISA detection kits that combine ease of use with high levels of specificity and sensitivity. Total NF-κB is accurately detected using Rockland's anti-RelA (p65) antibody, NF-κB p65 positive control lysate and NF-κB specific competitor dsDNA. At the core of this technology is Rockland's anti-RelA (p65) antibody - a thoroughly validated reagent that in over ten years of widespread use has been cited in numerous peer reviewed scientific publications.

Introduction

The NF-κB/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-κB [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-κB 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 κB sites, thereby regulating expression of target genes. In most cells, RelA/ NF-κB transcription complexes are present in an inactive form in the cytoplasm, bound to an inhibitor IκB. Certain stimuli result in the phosphorylation, ubiquitination and subsequent degradation of IκB proteins thereby enabling translocation of NF-κB into the nucleus[3]. The most common RelA/NF-κB dimer in mammals contains p50-RelA (p50/p65) heterodimers and is specifically called NF-κB. One of the target genes activated by NF-κB is that encoding IκBα. This feedback mechanism allows newly-synthesized IκBα to enter the nucleus, remove NF-κB from DNA and transport it back to the cytoplasm thereby restoring its inactive state. The importance of RelA/NF- κB transcription factors in human inflammation and certain diseases makes them attractive targets for potential therapeutics[4-6]. Rockland's NF-κB (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-κB response element is immobilized onto the bottom of wells of a 96 well plate (see Figure 1). NF-κB contained in a nuclear extract specifically binds to the NF-κB response element. NF-κB (p65) is detected by addition of a specific primary antibody directed against NF-κB (p65). A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450nm. Rockland's NF-κB (p65) Transcription Factor Assay detects human NF-κB (p65) and does not cross-react with NF-κB (p50).

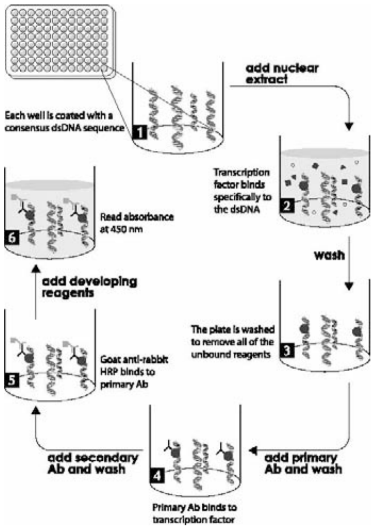

Figure 1. Schematic of the anti-RelA/ NF-κB (p65) Transcription Factor binding assay.

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Methods

The NF-κB (p65) antibody based immunometric transcription factor assay kit includes an easy to understand User's Manual with simple, straightforward instructions for the accurate collection of data. The manual describes general user precautions, storage and stability, a detailed description of the contents of the kit and a list of additional equipment/items required to run the assay (i.e. an ELISA plate reader capable of measuring absorbance at 450nm, adjustable and repeat micro pipettes, molecular biology grade water, DTT and purified cellular nuclear extracts). A protocol for the purification of nuclear extracts is also included. Typically this procedure is used for a 15ml cell suspension grown in a T75 flask or adherent cells grown to 80-90% confluence on a 100mm dish. As a general approximation, 107 cells yield 50µg of nuclear protein. The addition of protease and/or phosphatase inhibitors is recommended as proteins in nuclear extracts are labile. The kit includes most buffers and specifies appropriate storage conditions for individual components. Some buffers included require dilution to working concentrations prior to use.

#	Item	Storage
1	Transcription Factor Binding Assay Buffer (4X)	4°C
1a	Transcription Factor Reagent A	-20°C
2	Transcription Factor NF-κB (p65) Positive Control	-80°C
3	Transcription Factor Antibody Binding Buffer (10X)	4°C
4	Transcription Factor NF-κB (p65) Primary Antibody	-20°C
5	Wash Buffer Concentrate (400X)	4°C
5a	Tween 20	RT
6	Transcription Factor NF-κB Specific Competitor dsDNA	-20°C
7	HRP Goat Anti-Rabbit Secondary Antibody Conjugate	-20°C
8	Transcription Factor NF-κB 96 Well Plate	4°C
9	Plate Cover	N/A
10	Transcription Factor Developing Solution	4°C
11	Transcription Factor Stop Solution	4°C

Protocol

1. Bind active NF-κB (p65) to the consensus sequence
2. Add Anti-NF-κB (p65) Primary Antibody
3. Add HRP Goat anti-Rabbit conjugated Secondary Antibody
4. Develop and Read the Plate

Results

Accurate, sensitive and reproducible results are achieved that allow for the straightforward quantification of total NF-κB (p65) present in nuclear extracts. Data collected is sufficient for the generation of a standard curve (see Figure 2). Generally, EGTA and EDTA concentrations below 0.5mM do not affect the assay; however, ZnCl2 should be avoided at any concentration. Low concentrations of DTT or DMSO also do not affect results. A detailed troubleshooting guide is including to assisting the most novice user to achieve impressive results.

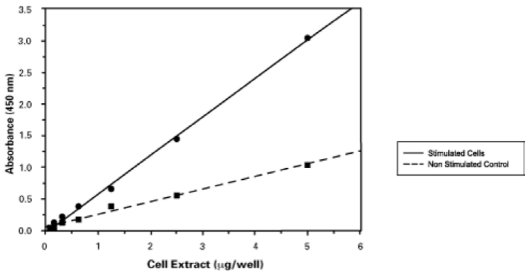

Figure 2. Assay of cell lysates isolated from stimulated (20ng/ml TNFκ for 30min) and nonstimulated HeLa cells demonstrating NF-κB (p65) activity.

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Conclusions

The sensitivity and specificity of Rockland Immunochemical's anti-NF-κB (p65) antibody based immunometric Transcription Factor Assay kit in combination with its ease of use, especially the detailed assay procedures, make this product an excellent choice for both routine and high throughput assays for total NF-κB (p65). Rockland Immunochemicals is currently developing additional kits for the detection of activated (i.e. translocated to the nucleas) NF-κB by using antibodies to the p65 NLS (Nuclear Localization Sequence) and also antibodies to specific phosphorylation sites on the p65 molecule.

NOTE: THIS PRODUCT IS NOT INTENDED FOR DIAGNOSTIC USE IN HUMANS OR ANIMALS.

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Related Products

Code	Product Description
100-4164	Anti-NFκB (p50) (NFKB1) [Rabbit]
100-4165	Anti-NFκB (p65) (Rel A) [Rabbit]
100-4165N	Anti-NFκB (p65) (Rel A) N-TERMINAL SPECIFIC [Rabbit]
600-401-271	Anti-NFκB (p65) NLS specific [Rabbit]
100-401-264	Anti-NFκB (p65) (Rel A) phospho specific pS276 [Rabbit]
100-401-266	Anti-NFκB (p65) (Rel A) phospho specific pS529 [Rabbit]
100-4166	Anti-NFκB cRel [Rabbit]
100-4184	Anti-NFκB (p105) [Rabbit]
100-4185	Anti-NFκB cRel [Rabbit]
100-4167C	Anti-IκB alpha (Hu, Ms, Rt Specific) C-terminal [Rabbit]
100-4186	Anti-IκB beta (Hu, Ms, Rt Specific) [Rabbit]
100-401-219	Anti-IKK alpha [Rabbit]
100-401-220	Anti-IKK beta [Rabbit]
600-401-267	Affinity Purified anti-IKKe phospho specific pT501 [Rabbit]
600-401-273	Affinity Purified anti-KB-RAS [Rabbit]
K-025	NFκB Oligonucleotide

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