

**Datasheet for KAA064****NFkB p50 ELISA Kit****Overview**

|                      |                               |
|----------------------|-------------------------------|
| <b>Description:</b>  | NFkB (P50) ELISA Kit - KAA064 |
| <b>Item No.:</b>     | KAA064                        |
| <b>Size:</b>         | 1 Kit                         |
| <b>Applications:</b> | ELISA                         |
| <b>Reactivity:</b>   | Human                         |

**Product Details****Background:**

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

|                            |                                                                                  |
|----------------------------|----------------------------------------------------------------------------------|
| <b>Synonyms:</b>           | NF- $\kappa$ B Transcription Factor Kit, p50 kit, EIA kit, NF kappaB ELISA Assay |
| <b>Detection Kit Type:</b> | ELISA Kit                                                                        |

**Target Details**

|                        |                                                                                                                  |
|------------------------|------------------------------------------------------------------------------------------------------------------|
| <b>Reactivity:</b>     | Human                                                                                                            |
| <b>Relevant Links:</b> | <ul style="list-style-type: none"><li><a href="#">NF-<math>\kappa</math>B (P50) ELISA Kit Protocol</a></li></ul> |

## Application Details

|                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b> | ELISA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Application Note:</b>    | Rockland's NF- $\kappa$ B (p50) 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- $\kappa$ B response element is immobilized onto the bottom of wells of a 96 well plate (see Figure 1 on page 4). NF- $\kappa$ B contained in a nuclear extract specifically binds to the NF- $\kappa$ B response element. NF- $\kappa$ B (p50) is detected by addition of a specific primary antibody directed against NF- $\kappa$ B (p50). A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. Rockland's NF- $\kappa$ B (p50) Transcription Factor Assay detects human NF- $\kappa$ B (p50). It will not cross-react with NF- $\kappa$ B (p65). |
| <b>Assay Dilutions:</b>     | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>ELISA:</b>               | 1:100                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| <b>Other:</b>               | DOE: 2/28/2025                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |

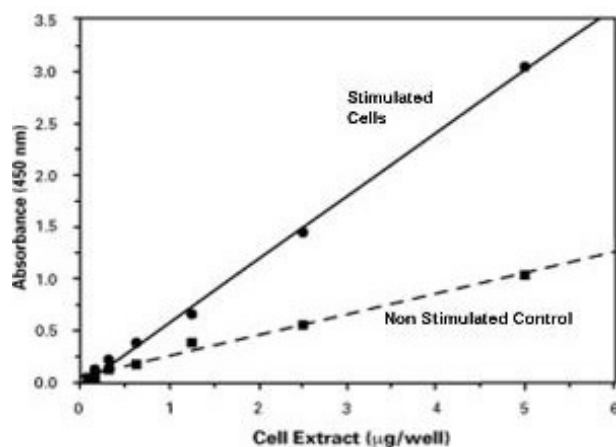
## Formulation

|                        |       |
|------------------------|-------|
| <b>Physical State:</b> | n/a   |
| <b>Concentration:</b>  | 1 Kit |

## Shipping & Handling

|                            |                                           |
|----------------------------|-------------------------------------------|
| <b>Shipping Condition:</b> | Wet Ice                                   |
| <b>Storage Condition:</b>  | See kit insert for complete instructions. |
| <b>Expiration:</b>         | See kit insert for complete instructions. |

## Images



### ELISA

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

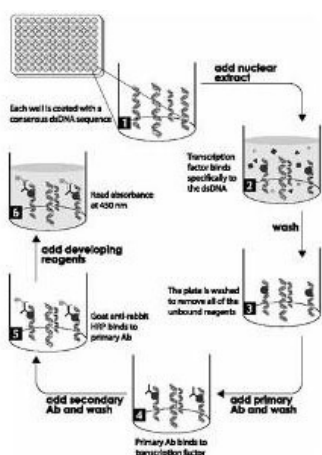
### Kit Box

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



### ELISA

Transcription Factor Binding Assay Schematic



## ELISA

### Sample Plate Format

|   | 1  | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|---|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A | U1 | U3  | U9  | U5  | U17 | U13 | U25 | U21 | U33 | U29 | U41 | U37 |
| B | U2 | U4  | U10 | U6  | U18 | U14 | U26 | U22 | U34 | U30 | U42 | U38 |
| C | U3 | U5  | U11 | U7  | U19 | U15 | U27 | U23 | U35 | U31 | U43 | U39 |
| D | U4 | U6  | U12 | U8  | U20 | U16 | U28 | U24 | U36 | U32 | U44 | U40 |
| E | U5 | U7  | U13 | U9  | U21 | U17 | U29 | U25 | U37 | U33 | U45 | U41 |
| F | U6 | U8  | U14 | U10 | U22 | U18 | U30 | U26 | U38 | U34 | PC  | PC  |
| G | U7 | U9  | U15 | U11 | U23 | U19 | U31 | U27 | U39 | U35 | Blk | Blk |
| H | U8 | U10 | U16 | U12 | U24 | U20 | U32 | U28 | U40 | U36 | CT  | CT  |

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

## Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.