ELISA Kit
Catalog #KHO0371

NF-κBp65
(Total)

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

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INTRODUCTION

The Nuclear Factor-kappa B (NF-κB) transcription factors comprise a group of protein dimers which are composed of various combinations of members of the NF-κB/Rel protein family. In mammals, there are at least five members of this protein family, including NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), c-Rel, RelA (p65), and RelB. In *Drosophila*, at least three members have been identified which are designated Dorsal, Dif, and Relish. All members of the NF-κB family share the Rel Homology Region (RHR), a domain of about 300 residues which mediates dimerization as well as DNA binding through the consensus sequence GGRNNYYCC. Within the Rel Homology Region is a subdomain designated the Nuclear Localization Sequence (NLS), which is involved in targeting NF-κB to the cell nucleus. While the RHR and NLS are common features of NF-κ B family members, only certain members possess a transcriptional activation domain, such as RelA (p65). Dimers containing the p65 subunit serve as potent activators of transcription, while homodimers composed of the NF-κB1 (p50) subunit, which lacks the transcriptional activation domain, may actually repress transcription. Among mammalian NF-κB's, the p65/p50 dimer is the most abundant.

Under resting conditions, NF-κB/Rel family members are sequestered in the cytoplasm in an inactive state through interaction with the IκB family members, which include the mammalian proteins IκBα, IκBβ (with its splice variants IκBβ1 and IκBβ2), IκBγ, IκBε, and Bcl-3, plus the precursors of NF-κB p105, and p100. This sequestration occurs through the interaction of IκBs ankyrin repeats with the RHR of the NF-κB transcriptional factors, an interaction that masks the Nuclear

Localization Sequences. The most abundant mammalian IκBs include IκBα, IκBβ1, IκBβ2, and IκBε.

The transport of NF-κB/Rel family members into the nucleus requires their liberation from the IκBs. The series of events leading to the liberation of NF-κB from IκBα includes the phosphorylation of specific serine residues IκBα (serines 32 and 36) located near the amino terminus. IκBα is then marked by its polyubiquitination, and finally translocated to the 26S proteasome for degradation. The kinase responsible for the initial phosphorylation of IκBα, IKK, is a protein kinase complex with M_r=700 kDa composed of two kinase subunits designated IKKα (M_r=85 kDa) and IKKβ (M_r=87 kDa), a regulatory subunit designated IKKγ, which is also known as NEMO (M_r=48 kDa), and possibly several other components.

Diverse stimuli that activate NF-κB through the degradation of IκB include TNF-α, IL-1β, LPS, viral infection, and various stresses. Among the wide array of genes under the control of NF-κB, are IL-6, VEGF, VCAM-1, and HIV long terminal repeat. NF-κB's regulation of IκB transcription provides a mechanism for NF-κB's limiting its own signaling.

NF-κB mediates inflammatory responses, immune responses, responses to viral infections, cell division, and regulation of apoptosis, serving both as an anti-apoptotic signal as well as a pro-apopototic signal.

The Invitrogen NF-κBp65 (Total) ELISA is designed to detect and quantify the level of NF-κBp65 protein, independent of its phosphorylation state. Although performance characterization of this ELISA kit was done primarily on human cell lines, cross-reactivity of this kit with mouse cells was observed. Reactivity of this ELISA kit with other species is not assured.

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READ ENTIRE PROTOCOL BEFORE USE

PRINCIPLE OF THE METHOD

The Invitrogen NF-κBp65 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for NF-κBp65 (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing NF-κBp65, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the NF-κBp65 antigen binds to the immobilized (capture) antibody. After washing, a rabbit antibody, specific for NF-κBp65, is added to the wells. During the second incubation, this antibody serves as a detector by binding to the immobilized NF-κBp65 protein captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase-labeled Anti-Rabbit IgG (Anti-Rabbit IgG-HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess Anti-Rabbit IgG-HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of NF-κBp65 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
NF-κBp65 (Total) Standard: Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle
*Antibody Coated Well*s, 12x8 Well Strips.	1 plate
NF-κBp65 (Total) Detection Antibody. Contains 0.1% sodium azide; blue dye*; 11 mL per bottle.	1 bottle
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
HRP Diluent. Contains 3.3 mM thymol; yellow dye*; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle
*Stabilized Chromoge*n, *Tetramethylbenzidine (TMB)*; 25 mL per bottle.	1 bottle
*Stop Solutio*n; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3
* In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored *Standard Diluent Buffer*, *Detection Antibody*, and *HRP Diluent* to help monitor the addition of solution to the reaction well. This does not in any way interfere with the test results.	

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Cell extraction buffer (see Recommended Formulation, p. 12).
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.
9. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
4. If particulate matter is present, centrifuge or filter prior to analysis.
5. All standards, controls and samples should be run in duplicate.
6. Samples containing NF-κBp65 protein extracted from cells should be diluted with *Standard Diluent Buffer* at least 1:10. This dilution is necessary to reduce the matrix effect of the cell lysis buffer. SDS concentration should be less than 0.01% before adding to the plate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots**.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
14. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

PROCEDURE FOR EXTRACTION OF PROTEINS FROM CELLS AND NUCLEI

A. Recommended Formulation of Hypotonic Buffer

20 mM Tris-HCl, pH 7.4
10 mM NaCl
3 mM $MgCl_2$

B. Protocol for Nuclear Extract

This protocol has been successfully applied to several cell lines of human origin. Researchers should optimize the cell extraction procedures for their own applications.

1. Collect cells (5 x 10^6) in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet.
4. Transfer the cells into a pre-chilled microcentrifuge tube.
5. Gently resuspend cells in 500 µL 1x Hypotonic Buffer by pipetting up and down several times. Incubate for 15 minutes on ice.
6. Add 25 µL detergent (10% NP40) and vortex for 10 seconds at highest setting.
7. Centrifuge the homogenate for 10 minutes at 3000 rpm at 4°C.
8. Transfer and save the supernatant. This supernatant contains the cytoplasmic fraction. The pellet is the nuclear fraction.
9. Resuspend nuclear pellet in 50 µL Cell Extraction Buffer (see page 13) for 30 minutes on ice with vortexing at 10 minute internals.
10. Centrifuge for 30 minutes at high speed at 4°C. Transfer supernatant (nuclear fraction) to a clean microcentrifuge tube.

11. Aliquot and store at -80°C. The nuclear extracts are ready for assay.

C. Recommended Formulation of Cell Extraction Buffer:

10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
1% Triton X-100
10% glycerol
0.1% SDS
0.5% deoxycholate
1 mM PMSF (stock is 0.3 M in DMSO)
Protease inhibitor cocktail (e.g., Sigma Cat. # P-2714) (reconstituted according to manufacturer's guideline). Add 250 µL per 5 mL Cell Extraction Buffer.

This buffer is stable for 2 to 3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the Cell Extraction Buffer should be thawed on ice. Important: add the protease inhibitors just before using. The stability of protease inhibitor supplemented Cell Extraction Buffer is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

This buffer (minus protease inhibitor and PMSF) is available from Invitrogen (Cat. # FNN0011).

D. Protocol for Cell Extraction

This protocol has been successfully applied to several cell lines of human origin. Researchers should optimize the cell extraction procedures for their own applications.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at -80°C and lysed at a later date.)
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice, with vortexing at 10 minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of NF-κBp65. For example, 10^7 Jurkat cells grown in RPMI plus 10% FBS can be extracted in 1 mL of Extraction Buffer. Under these conditions, use of 1-5 µL of the clarified cell extract diluted to a volume of 100 µL/well in Standard Diluent Buffer (See **Assay Method**) is sufficient for the detection of NF-κB.
5. Transfer extract to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
6. Aliquot the clear lysate to clean microfuge tubes. These samples are ready for assay. Lysates can be stored at -80°C. Avoid multiple freeze/thaw cycles.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of NF-κBp65 (Total) Standard

Note: This NF-κBp65 (Total) Standard (lyophilized cell extract from HeLa cells) was calibrated against the mass of a purified, truncated, recombinant NF-κBp65 protein expressed in *E. coli*.

1. Reconstitute *NF-κBp65 (Total) Standard* with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 5,000 pg/mL NF-κBp65. Use standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 2500, 1250, 625, 312.5, 156.3, and 78.1 pg/mL NF-κBp65.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of NF-κBp65 (Total) Standard

Standard:	Add:	Into:
5000 pg/mL	Prepare as described in step 1	
2500 pg/mL	0.25 mL of the 5000 pg/mL std.	0.25 mL of the Diluent Buffer
1250 pg/mL	0.25 mL of the 2500 pg/mL std.	0.25 mL of the Diluent Buffer
625 pg/mL	0.25 mL of the 1250 pg/mL std.	0.25 mL of the Diluent Buffer
312.5 pg/mL	0.25 mL of the 625 pg/mL std.	0.25 mL of the Diluent Buffer
156.3 pg/mL	0.25 mL of the 312.5 pg/mL std.	0.25 mL of the Diluent Buffer
78.1 pg/mL	0.25 mL of the 156.3 pg/mL std.	0.25 mL of the Diluent Buffer
0 pg/mL	0.25 mL of the Diluent Buffer	An empty tube

Remaining reconstituted standard should be discarded or frozen at -80^{o}C for further use.

C. Storage and Final Dilution of Anti-Rabbit IgG HRP (100X)

Please Note: The *Anti-Rabbit IgG HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Anti-Rabbit IgG HRP (100X)* to reach room temperature. Gently mix. Pipette *Anti-Rabbit IgG HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Within 1 hour of use, dilute 10 µL of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-Rabbit IgG-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Anti-Rabbit IgG HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Anti-Rabbit IgG-HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 µL of standards, samples or controls to the appropriate microtiter wells. Samples prepared in Cell Extraction Buffer must be diluted 1:10 or greater in *Standard Diluent Buffer* (for example, 10 µL sample into 90 µL buffer). While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal. The dilution chosen should be optimized for each

experimental system. Tap gently on side of plate to thoroughly mix. (See **REAGENT PREPARATION AND STORAG**E, Section B.)

4. Cover wells with *plate cover* and incubate for **2 hours at room temperature**.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 µL of *NF-κBp65 (Total*) *Detection Antibod*y solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover wells with *plate cover* and incubate for **1 hour at room temperature.**
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 µL *Anti-Rabbit IgG HRP* Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover wells with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
13. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or**

metalized mylar. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

14. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
16. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the NF-κBp65 concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by dilution factor to correct for the dilution in step 3.** (Samples producing signals higher than the highest standard (5 ng/mL) should be further diluted in

Standard Diluent Buffer and reanalyzed, multiplying the concentration by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 5000 pg/mL NF-κBp65.

Standard NF-κBp65 (pg/mL)	Optical Density (450 nm)
0	0.182
78.1	0.259
156.3	0.320
312.5	0.445
625	0.662
1250	1.112
2500	1.817
5000	2.852

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 5 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >5 ng/mL with *Standard Diluent Buffer;* reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native NF-κBp65 in various matrices has not been investigated. Although NF-κBp65 degradation in the Cell Extraction Buffer described in this protocol has not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity of this assay is <50 pg/mL of NF-κBp65. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. The sensitivity of this ELISA was compared to Western blotting using known quantities of NF-κBp65. The data presented in Figure 1 show that the sensitivity of the ELISA is approximately 4x greater than that of Western blotting. The bands shown in the Western blotting data were developed using mouse monoclonal NF-κBp65 antibody, an alkaline phosphatase conjugated anti-mouse IgG followed by chemiluminescent substrate and autoradiography.

Figure 1: Comparison of NF-κBp65 detection by ELISA and Western Blot:

NF-κBp65 (65 kDa)								
ELISA: (O.D. 450 nm)	2.852	1.817	1.112	0.662	0.445	0.320	0.259	0.182
NF-κBp65 (pg/test)	500	250	125	62.5	31.3	15.7	7.8	0

PRECISION

1. Intra-Assay Precision

Samples of known NF-κBp65 concentration were assayed in replicates of 12 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1833.6	886.4	305.1
SD	153.3	68.5	17.7
%CV	8.4	7.7	5.8

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 36 times in multiple assays to determine precision between assays.

RECOVERY

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	1855.6	868.2	299.4
SD	194.2	85.4	32.7
%CV	10.4	9.8	10.9

SD = Standard Deviation
CV = Coefficient of Variation

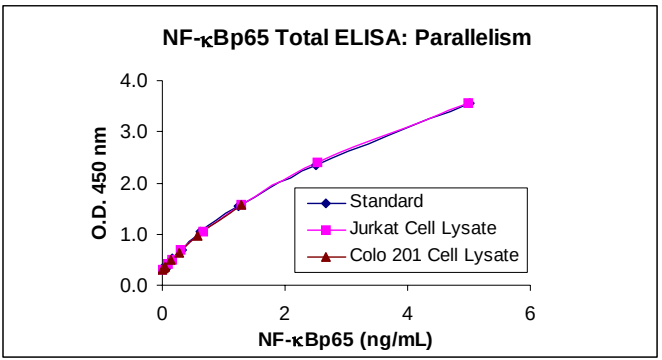
To evaluate recovery, extraction buffer was diluted 1:10 with *Standard Diluent Buffer* to bring the SDS concentration to <0.01%. NF-κBp65 Standard was spiked into the cell extraction buffer. On average, 85% recovery was observed.

PARALLELISM

Natural NF-κBp65 from extracts of Jurkat and Colo201 cells cultured in RPMI 1640 (Cat.# P107-500) containing 10% FBS were serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the NF-κBp65 standard curve. Parallelism demonstrated in Figure 2 indicates that the standard accurately reflects full length NF-κBp65 content in samples.

Figure 2

LINEARITY OF DILUTION



Jurkat cells were grown in tissue culture medium containing 10% FBS and lysed with Cell Extraction Buffer. This lysate was diluted in *Standard Diluent Buffer* over the range of the assay and measured for NF-κBp65 content. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

	Extraction Buffer		
Dilution	**Measured (pg/mL)**	**Expected (pg/mL)**	**% Expected**
Neat	2526	2526	100
1/2	1287	1263	102
1/4	663	632	105
1/8	310	316	98
1/16	151	158	96
1/32	87	79	110

SPECIFICITY

The NF-κBp65 (Total) ELISA kit is specific for measurement of total NF-κBp65. To determine the specificity of this ELISA kit, cell extracts from different cell lines, each at a concentration of 200 µg/mL total protein, were analyzed. The data presented in Figure 3 show that the NF-κBp65 total ELISA detects NF-κBp65 in cell lysates from human Jurkat, Colo201, HT1080, CEM and HeLa cells. The levels of NF-κ Bp65 protein detected with this ELISA are consistent with results obtained by Western blot analysis (insert).

The assay was found to have no cross-reactivity with either NF-κBp50 or IκBα. Cross-reactivity with other NF-κB family members was not evaluated.

Figure 3

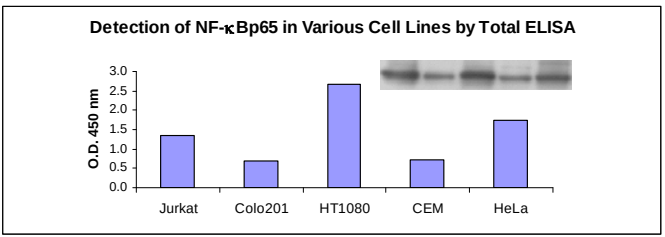
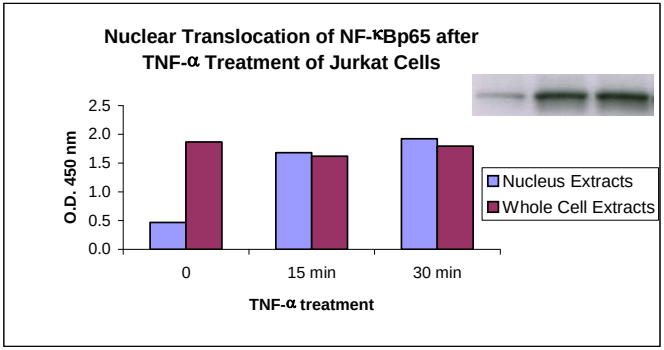


Figure 4



Jurkat cells (5 x 10^6) were treated with TNF-α at 20 ng/mL for 15 and 30 minutes. The cells were harvested, resuspended in Hypotonic Lysis Buffer, and centrifuged to isolate nuclei. The nuclei were lysed with Extraction Buffer and the resulting lysates were tested by ELISA and Western blot. The data show that the NF-κBp65 (Total) ELISA kit can monitor and quantitate p65 translocation into the nucleus, which is consistent with Western blot analysis (insert).

REFERENCES

1. Adams, J. (2001) Proteasome inhibition in cancer: development of PS-341. Semin. Oncol. 28(6):613-619.

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

NF-κBp65 (Total) Assay Summary