

# ab207220 NFkB p52 Transcription Factor Assay Kit (Chemiluminescent)

Instructions for use:

For quantitative measurement of NFkB p52 activation in human and mouse nuclear extracts.

This product is for research use only and is not intended for diagnostic use.

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#### INTRODUCTION

#### 1. BACKGROUND

NFkB p52 Transcription Factor Assay Kit (Chemiluminescent) (ab207220) is a high throughput assay to quantify NFkB p52 activation. This assay combines a quick ELISA format with a sensitive and specific non-radioactive assay for transcription factor activation.

A specific double stranded DNA sequence containing the NFkB p52 consensus binding site (5' – GGGACTTTCC – 3') has been immobilized onto a 96-well plate. Active NFkB p52 present in nuclear extracts specifically binds to the oligonucleotide. NFkB p52 is detected by a primary antibody that recognizes an epitope of NFkB p52 accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides a sensitive chemiluminescent readout that can be quantified using by luminescence. This product detects only human and mouse NFkB p52.

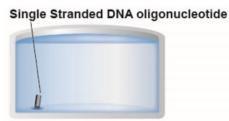
#### Key performance and benefits:

- Assay time: 3.5 hours (cell extracts preparation not included).
- Detection limit: < 40 ng nuclear extract/well.</li>
- Detection range: 0.039 2.5 μg nuclear cell extract/well.

The transcription factor NFkB is implicated in the regulation of many genes that code for mediators of the immune, acute phase and inflammatory responses. NFkB is composed of homo- and heterodimeric complexes of members of the Rel (NFkB) family. There are five subunits of the NFkB family in mammals: p50, p65 (RelA), c-Rel, p52 and RelB. These proteins share a conserved 300 amino acid sequence in the N-terminal region, known as the Rel homology domain, that mediates DNA binding, protein dimerization and nuclear localization. Various dimer combinations of the NFkB subunits have distinct DNA binding specificities and may serve to activate specific sets of genes such as adhesion molecules, immunoreceptors and cytokines. Proteolytic cleavage of p102 generates the mature NFkB p52 subunit. The p52 homodimers are, in general, repressors of kB site transcription, but they also bind to the nuclear protein Bcl3, and such complexes can function as transcriptional activators.

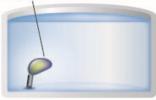
# INTRODUCTION

#### 2. ASSAY SUMMARY



Prepare all reagents, nuclear extracts and controls as instructed. Plate is supplied precoated with an oligonucleotide containing NFkB p52 consensus binding site.

#### Sample



Add sample (nuclear extracts containing activated transcription factor) to appropriate wells. Incubate plate for 1 hour at RT.

#### Primary Antibody



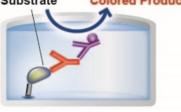
Add primary antibody to wells. Incubate plate for 1 hour at RT.

**HRP-Conjugated Antibody** 



Aspirate and wash each well. Add HRP-conjugated secondary antibody. Incubate plate for 1 hour at RT.





Aspirate and wash each well. Add Chemiluminescent Reagent.

Measure signal immediately using a luminometer or CCD camera system

#### 3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances.
   However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

#### 4. STORAGE AND STABILITY

Store kit at -20°C (nuclear extract that must be kept at -80°C) in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt. After first use, components are stable for 6 months.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

#### 5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

#### 6. MATERIALS SUPPLIED

Item	Amount		Storage Condition	Storage Condition	
	1 Plate	5 Plates	(Before Preparation)	(After Preparation)	
NFkB p52 antibody	11 µL	55 µL	-20°C	4°C	
Anti-rabbit HRP- conjugated IgG (0.25 µg/µL)	11 μL	55 μL	-20°C	4°C	
Wild-type oligonucleotide (10 pmol/µL)	100 μL	500 μL	-20°C	-20°C	
Mutated oligonucleotide (10 pmol/µL)	100 μL	500 μL	-20°C	-20°C	
Raji nuclear extract (2.5 µg/µL)	40 μL	200 µL	-80°C	-80°C	
Dithiothreitol (DTT)	100 µL	500 μL	-20°C	-20°C	
Protease Inhibitor Cocktail	100 μL	500 μL	-20°C	-20°C	
Herring sperm DNA (1 μg/μL)	100 µL	500 μL	-20°C	-20°C	
Lysis Buffer	10 mL	50 mL	-20°C	4°C	
Binding Buffer	10 mL	50 mL	-20°C	4°C	
10X Wash Buffer	22 mL	110 mL	-20°C	4°C	
10X Antibody Binding Buffer	2.2 mL	11 mL	-20°C	4°C	
Chemiluminescent Reagent	2 mL	10 mL	-20°C	4°C	
Reaction Buffer	4 mL	20 mL	-20°C	4°C	
96-well NFkB chemi assay plate	1	5	-20°C	4°C	
Plate sealer	1	5	-20°C	RT	

### 7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate luminometer or CCD camera-couple imaging system
- MilliQ water or other type of double distilled water (ddH<sub>2</sub>O)
- · Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Rocking Platform

#### For nuclear extract preparation:

- Hypotonic buffer (20 mM Hepes pH7.5, 5 mM NaF, 10 μM Na<sub>2</sub>MoO<sub>4</sub>, 0.1 mM EDTA)
- Phosphatase Inhibitors (NaF, β-glycerophosphatase, PNPP, NaVO<sub>3</sub>)
- 10X PBS (0.1 M phosphate buffer pH7.5, 1.5 M NaCl, 27 mM KCl)
- NP-40

Alternatively, you can use our Nuclear Extraction Kit (ab113474) to prepare nuclear extracts.

#### 8. TECHNICAL HINTS

- This kit is sold based on number of tests. A 'test' simply refers
  to a single assay well. The number of wells that contain sample
  or control will vary by product. Review the protocol completely
  to confirm this kit meets your requirements. Please contact our
  Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

#### 9. REAGENT PREPARATION

Briefly centrifuge small vials at low speed prior to opening.

Please see Quick Table for Reagent Preparation at the end of this section for a quick reference.

#### 9.1. Dithiothreitol (DTT, 1 M):

Ready to use as supplied. Dilute in Lysis Buffer and Binding Buffer as described in section 9.4 and 9.5 respectively. Store at - 20°C.

#### 9.2. Protease Inhibitor Cocktail (PIC):

Ready to use as supplied. Dilute in Lysis Buffer as described in section 9.4. Store at -20°C.

#### 9.3. Herring sperm DNA (1 μg/μL):

Ready to use as supplied. Dilute in Binding Buffer as described in section 9.5. Store at -20°C.

#### 9.4. Lysis Buffer:

Prepare Complete Lysis Buffer (CLB) by adding 5  $\mu$ L of 1 M DTT and 10  $\mu$ L of Protease Inhibitor Cocktail to 1 mL of Lysis Buffer – see Quick Table for Reagent Preparation to see how much is required depending on number of tests. Use the CLB immediately for cell lysis. The remaining amount should be discarded if not used in the same day.

Store undiluted Lysis Buffer at 4°C.

# 9.5. **Binding Buffer:**

Prepare Complete Binding Buffer (CBB) by adding 2  $\mu$ L of 1 M DTT and 10  $\mu$ L of 1  $\mu$ g/ $\mu$ L Herring Sperm DNA to 1 mL of Binding Buffer – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Discard remaining CBB if not used in the same day. Store undiluted Binding Buffer at 4°C.

#### 9.6. Wash Buffer:

Prepare **1X Wash Buffer** by making a 1/10 dilution of 10X Wash Buffer in distilled water  $(ddH_2O)$  – see Quick Table for Reagent Preparation to see how much is required depending on number of tests. Mix gently to avoid foaming.

1X Wash Buffer can be stored at 4°C for one week. **NOTE**: Tween 20 contained in the 10X Wash Buffer may form clumps. If this happens, homogenize buffer by vortexing for 2 minutes prior to use.

Store undiluted 10X Wash Buffer at 4°C.

#### 9.7. Antibody Binding Buffer:

Prepare **1X Antibody Binding Buffer (ABB)** by making a 1/10 dilution of 10X Antibody Binding Buffer in distilled water (ddH<sub>2</sub>O) – see Quick Table for Reagent Preparation to see how much is required depending on number of tests. Mix gently to avoid foaming.

Discard remaining 1X ABB if not used in the same day. **NOTE:** BSA contained in the 10X Antibody Binding Buffer may form clumps. If this happens, homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use.

Store undiluted 10X Antibody Binding Buffer at 4°C.

# 9.8. NFkB p52 Antibody:

Dilute supplied NFkB p52 antibody 1/1000 in 1X ABB – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Aliquot and store undiluted NFkB p52 antibody at 4°C. Avoid multiple freeze/thaw cycles.

#### 9.9. Anti-rabbit HRP-conjugated Antibody (0.25 μg/μL):

Dilute supplied anti-rabbit HRP-conjugated antibody 1/500 in 1X ABB. Prepare a further 1/20 dilution in 1X ABB to achieve a 1/10,000 final antibody dilution – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Aliquot and store undiluted anti-rabbit HRP-conjugated antibody at 4°C. Avoid multiple freeze/thaw cycles.

#### 9.10. Chemiluminescent Reagent:

Equilibrate Chemiluminescent Reagent to room temperature 1 hour prior to use.

Prepare Chemiluminescent Working Solution by making a 1/2 dilution of Chemiluminescent Reagent in Reaction Buffer – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

The Chemiluminescent Working Solution is stable for several hours. After the Chemiluminescent Working Solution is aliquoted into the wells, discard the remaining solution.

# 9.11. Raji nuclear extract (2.5 μg/μL):

Ready to use as supplied. Extract has been optimized to be used at 1.25  $\mu$ g/well. There is enough extract to perform 80 reactions per plate. Aliquot extract in 5  $\mu$ L fractions and store at -80°C. Avoid multiple freeze/thaw cycles.

#### 9.12. Control oligonucleotides (wild-type & mutated):

Oligonucleotides are provided to monitor the specificity of the assay.

Wild-type oligonucleotide: competes with sample nuclear extracts for NFkB consensus binding site.

Mutated oligonucleotide: no effect on ability of sample nuclear extracts to bind to NFkB consensus binding site.

Use wild-type and/or mutated oligonucleotide at 20 pmol/well: dilute 2  $\mu$ L appropriate oligonucleotide in 31.8  $\mu$ L of CBB (section 9.5) per well used — see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Aliquot undiluted oligonucleotides and store at -20°C. Avoid multiple freeze/thaw cycles.

#### 9.13. 96-well assay plate:

Ready to use as supplied.

Store unused strips in the aluminium pouch at 4°C.

#### 9.14. Plate sealer:

Ready to use as supplied. Store at room temperature.

# **Quick Table for Reagent Preparation**

Reagents to prepare	Components	1 well	1 strip (8 wells)	6 strips (48 wells)	12 strips (92 wells)
Complete	DTT	0.11 μL	0.9 µL	5.4 µL	10.8 µL
Lysis Buffer	PIC	0.23 μL	1.8 µL	10.8 μL	21.6 µL
(CLB)	Lysis Buffer	22.2 µL	177.3 μL	1.064 mL	2.128 mL
	TOTAL REQUIRED	22.5 µL	180.0 μL	1.08 mL	2.16 mL
Complete	DTT	0.07 μL	0.54 μL	3.2 µL	6.5 µL
Binding Buffer (CBB)	Herring Sperm DNA	0.34 µL	2.7 μL	16.2 µL	32.4 µL
(022)	Binding Buffer	33.4 µL	267 µL	1.6 mL	3.2 mL
	TOTAL REQUIRED	33.8 µL	270 μL	1.62 mL	3.24 mL
Oligo	Wt or	2 µL	16 µL	96 µL	N/A
(wt or	mutated oligo				
mutated) in CBB	CBB	31.8 µL	254 µL	1.524 mL	N/A
ODD	TOTAL REQUIRED	33.8 µL	270 μL	1.62 mL	N/A
1X Wash	ddH <sub>2</sub> O	2.025 mL	16.2 mL	97.2 mL	194.4 mL
Buffer	10X Wash Buffer	225 μL	1.8 mL	10.8 mL	21.6 mL
	TOTAL REQUIRED	2.25 mL	18 mL	108 mL	216 mL

Reagents to prepare	Components	1 well	1 strip (8 wells)	6 strips (48 wells)	12 strips (92 wells)
1X Ab	ddH <sub>2</sub> O	157.5 μL	1.26 mL	7.56 mL	15.12 mL
Buffer*	10x ABB	17.5 µL	140 µL	840 µL	1.68 mL
	TOTAL REQUIRED	175 µL	1.4 mL	8.4 mL	16.8 mL
1° Ab	NFkB p52 Ab	0.055 μL	0.44 µL	2.64 µL	5.06 µL
1/1000 Dillution	1X ABB	55 µL	440 µL	2.64 mL	4.95 mL
Dillution	TOTAL REQUIRED	55.06 μL	440.4 µL	2.64 mL	4.96 mL
2° Ab	HRP-conj Ab	0.1 µL	0.8 µL	4.8 µL	9.6 µL
pre-dilution (1/500)	1X ABB	50 µL	400 µL	3.195 mL	4.79 mL
(1/300)	TOTAL REQUIRED	50.1 μL	400.8 μL	3.2 mL	4.8 mL
2° Ab 1/20 Dilution	Pre-diluted HRP-conj Ab	2.75 μL	22 µL	132 µL	253 μL
	1X ABB	52.25 μL	418 µL	2.51 mL	4.81 mL
	TOTAL REQUIRED	55 µL	440 µL	2.64 mL	5.06 mL
Chemi-lumi Working	Chemi Reagent	18.7 µL	150 µL	0.9 mL	1.8 mL
Reaction	Reaction Buffer	37.5 μL	300 µL	1.84 mL	3.6 mL
	TOTAL REQUIRED	56.2 μL	450 μL	2.7 mL	5.4 mL

<sup>\*</sup>Volumes listed refer to preparation of buffer for diluting both primary and secondary antibodies.

#### 10. SAMPLE PREPARATION

- We recommend using our Nuclear Extraction Kit (ab113474) to prepare nuclear extracts, as it contains all necessary buffers and will help to reduce inconsistencies in the assay that may arise from using homemade or other buffers.
- Alternatively, you can refer to the protocol below.

#### 10.1. Prepare reagents needed:

10X PBS	For 250 mL:
0.1 M Phosphate Buffer, pH 7.5	3.55g Na <sub>2</sub> HPO <sub>4</sub> +
	0.61g KH <sub>2</sub> PO <sub>4</sub>
1.5 M NaCl	21.9 g
27 mM KCl	0.5 a

Adjust to 250 mL with  $ddH_2O$ . Prepare a 1X PBS solution dilute 10X PBS solution 1/10 in  $ddH_2O$ .

Sterilize 1X PBS pH 7.5 solution by filtering through a 0.2  $\mu$ m filter. Store filter-sterilized solution at 4°C.

PIB (Phosphatase Inhibitor Buffer)	For 10 mL:	
125 mM NaF	52 mg	
250 M β-glycerophosphate	0.55 g	
250 mM PNPP	1.15 g	
25 mM NaVO <sub>3</sub>	31 mg	

Adjust to 10 mL with  $ddH_2O$ . Mix the chemical by vortexing. Incubate solution at 50°C for 5 minutes. Mix again. Store at -20°C.

HB (Hypotonic Buffer)	For 50 mL:
20 mM Hepes, pH 7.5	0.24 g
5 mM NaF	12 mg
10 μM Na₂MoO₄	5 μL of 0.1 l

10 μM Na2MoO45 μL of 0.1 M solution0.1 mM EDTA10 μL of 0.5 M solution

Adjust pH to 7.5 with 1 N NaOH. Adjust volume to 50 mL with  $ddH_2O$ . Sterilize by filtering through a 0.2  $\mu$ m filter. Store filter-sterilized solution at 4°C.

**PBS/PIB:** prior to use, add 0.5 mL of PIB to 10 mL of 1X PBS.

- 10.2. Use cells from a confluent 100-mm dish / 75 cm<sup>2</sup> flask. 1 x 10<sup>7</sup> cells yield approximately 0.5 mg of nuclear extract.
- 10.3. Wash cells with 10 mL of ice-cold PBS/PIB solution.
- 10.4. Add 10 mL of ice-cold PBS/PIB and scrape cells off the dish with a cell scraper. Transfer cells to a pre-chilled 15 mL tube and centrifuge at 300 x g for 5 minutes at 4°C in a pre-chilled centrifuge. Discard supernatant.
- 10.5. Resuspend pellet in 1 mL of ice-cold HB buffer by gently pipetting and transfer the cells into a pre-chilled 1.5 mL tube.
- 10.6. Allow cells to swell on ice for 15 minutes.
- 10.7. Add 5  $\mu$ L 10% NP-40 (0.5% final) and mix by gently pipetting.
- 10.8. Centrifuge homogenate for 30 seconds at 4°C in a microcentrifuge. Discard supernatant.
- 10.9. Resuspend nuclear pellet in 50  $\mu$ L Complete Lysis Buffer (see section 9.4) and rock the tube gently on ice for 30 minutes on a shaking platform.
- 10.10. Centrifuge for 10 minutes at 14,000 x g at 4°C and save supernatant (nuclear extract).
- 10.11.Determine protein concentration of the extract by using a Bradford-based assay. We recommend BCA Protein Quantification Kit (ab102536).
- 10.12.Aliquot and store nuclear extracts at -80°C. Avoid multiple freeze/thaw cycles.

#### **ASSAY PROCEDURE**

#### 11. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to correct temperature prior to use.
- We recommend to assay all controls and samples in duplicate.
- Prepare all reagents and samples as directed in the previous sections.
- If less than 8 strips are used, cover the unused wells with a
  portion of the plate sealer while performing the assay. The
  control of these wells is stable at RT if kept dry and can
  therefore be used later for a separate assay. Use strip holder
  for the assay.

#### 11.1. NFkB p52 binding to its consensus sequence

- 11.1.1. Add 30 µL CBB containing wt or mutated oligo (Step 9.12) to the Competitive Binding Control wells.
- 11.1.2. Add 30 µL CBB to each of the other wells.
- 11.1.3. Prepare sample and control wells:

Competitive Binding control wells =  $20 \mu L$  of sample diluted in CLB (use  $0.2 - 2 \mu g$  of nuclear extract/well).

**Sample wells** =  $20 \mu L$  of sample diluted in CLB (use  $0.2 - 2 \mu g$  of nuclear extract/well).

**Positive control wells =**  $0.5 \, \mu L$  of provided nuclear extract +  $19.5 \, \mu L$  CLB ( $1.25 \, \mu g$  nuclear extract per well).

Blank wells = 20  $\mu$ L CLB only.

- 11.1.4. Use the provided adhesive cover to seal the plate. Incubate for 1 hour at RT with mild agitation (100 rpm on a rocking platform).
- 11.1.5. Wash each well 3 times with 200 µL 1X Wash Buffer. For each wash, flick plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

#### **ASSAY PROCEDURE**

#### 11.2. Primary antibody binding

- 11.2.1. Add 50  $\mu$ L of diluted NFkB p52 antibody (1/1000 in 1X ABB) to all wells being used.
- 11.2.2. Cover plate and incubate for 1 hour at RT without agitation.
- 11.2.3. Wash each well 3 times with 200 µL 1X Wash Buffer. For each wash, flick plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

#### 11.3. Secondary antibody binding

- 11.3.1. Add 50 μL diluted anti-rabbit HRP-antibody (1/10,000 dilution in 1X ABB) to all wells being used.
- 11.3.2. Cover plate and incubate for 1 hour at RT without agitation.
- 11.3.3. During this incubation, place Chemiluminescent Reagent at RT.
- 11.3.4. Wash each well 4 times with 200 µL 1X Wash Buffer. For each wash, flick plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels

#### 11.4. Measurement

- 11.4.1. Add 50 µL RT Chemiluminescent Working Solution to all wells being used. Minimize exposure of the plate to light. Pop any bubbles that remain in the wells to ensure accurate measurements and then read the plate immediately.
- 11.4.2. Read chemiluminescence using a luminometer or CCD camera system. You may need to customize the plate geometry to work with your plate reader.

Plate length =  $127,890 \mu m$  Plate height =  $15,000 \mu m$ 

Plate width =  $85,500 \mu m$  Height tolerance =  $500 \mu m$ 

#### 12. QUICK ASSAY PROCEDURE

**NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

#### Sample binding to consensus sequence

- Add 30 μL CBB + wt/mutant oligo to Competitive Binding control wells. Add 30 μL CBB to each of the other wells used.
- Add 20 µL sample, positive control and blank to the relevant wells.
- Incubate 1 hour RT with mild agitation (100 rpm on a rocker).
- Wash each well 3 times with 200 µL 1X Wash Buffer.



#### Primary antibody binding

- Add 50 μL diluted primary antibody (1/1000) to each well.
- Incubate 1 hour RT with no agitation.
- Wash each well 3 times with 200 μL 1X Wash Buffer.



# Secondary antibody binding

- Add 50 μL diluted HRP antibody (1/10,000) to each well.
- Incubate 1 hour RT with no agitation.
- Wash each well 4 times with 200 μL 1X Wash Buffer.



#### Measurement

- Add 50 µL RT Chemiluminescent Working Solution to each well.
- Read chemiluminescence immediately.

# 13. TROUBLESHOOTING

Problem	Cause	Solution
No signal or weak signal in any well	Omission of key reagent	Check that all reagents have been added in the correct order
	Substrate or conjugate in no longer active	Test conjugate and substrate for activity
	Enzyme inhibitor present	Sodium azide will inhibit peroxidase reaction; do not add to buffers
	Plate reader or CCD camera settings not optimal	Verify plate reader is set for luminescence measurement mode or check filter settings on the CCD camera
	Incorrect assay temperature	Bring Reagents to room temperature
No Signal or weak signal in sample wells	Not enough nuclear extract per well	Increase amount of nuclear extract – do not exceed 40 µg/well
	NFkB p52 is poorly activated or inactivated in nuclear fractions	Perform a time course for NFkB p52 activation in the studied cell line
	Nuclear extracts are not from correct species	This product specifically detects bound NFkB p52 in human and mouse samples
	Salt concentration too high in binding reaction	Reduce amount of extract per well or dialyze before use
High background in all wells	Measurement time too long	Reduce integration or exposure time on luminometer or CCD camera
	Concentration of antibodies too high	Increase antibody dilutions
	Inadequate washing	Ensure all wells are filled with Wash Buffer and follow washing recommendations
High background in sample wells	Too much nuclear extract per well	Decrease amount of nuclear extract to 0.01-0.05 μg/well
	Concentration of antibodies is too high	Perform antibody titration to determine optimal concentration. Start using 1/2000 for 1ry Ab and 1/50000 for the 2ry Ab. Assay sensitivity will be decreased

### 14. INTERFERENCES

These chemicals or biological materials will cause interference in this assay causing compromised results or complete failure:

• Sodium azide – it will inhibit the peroxidase reaction. Do not add to any buffer to be used in this assay.

# **15. NOTES**



#### **Technical Support**

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For all technical or commercial enquiries please go to:

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