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NF-κB Activation: A Molecular Link Between Chronic Inflammation and Cancer

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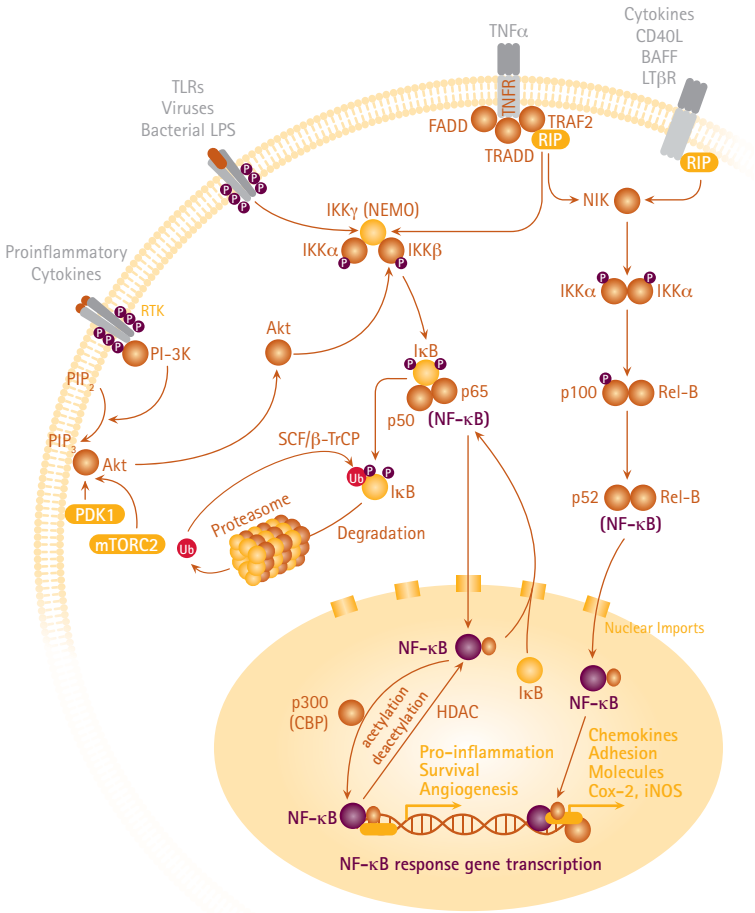
Inflammation, a normal physiological response to injury or infection, isolates the damaged area, mobilizes effector cells and molecules to the site of damage, and ultimately promotes healing. The inflammatory response includes transcriptional activation of several pro-inflammatory genes, which leads to the release of pro-inflammatory cytokines and chemokines, adhesion molecules, and reactive oxygen and nitrogen species. Simultaneously, anti-inflammatory pathways are also activated that serve as counter regulatory measures to keep the inflammatory response in check. The inflammatory response can be either acute or chronic. Acute inflammation may last only for a few days and can be easily treated with non-steroidal anti-inflammatory drugs (NSAID). However, chronic inflammation can last for several weeks or months and can cause severe tissue damage.



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The Good and the Bad Side of Inflammatory Response

Production and release of free radicals at the site of inflammation can be helpful in eliminating invading pathogens and foreign bodies, but these free radicals can also damage healthy epithelial and stromal cells, which may eventually lead to malignancy. Inflammation resulting from chronic exposure to infectious and non-infectious agents is an important factor in tumor development. In addition, excessive lipid peroxidation, protein nitration, and DNA damage caused by free radicals can activate pathways that lead to the transcriptional activation of several proto-oncogenes. Certain pro-inflammatory cytokines that are encoded by the target genes of nuclear factor κB (NF-κB) are also linked to tumor development and progression. The inflammatory link to carcinogenesis is also supported by the fact that the NF-κB signal transduction pathway is shown to be dysregulated in a variety of human cancers, especially those of lymphoid cell origin. Several human lymphoid cancer cells are reported to have either mutations or amplifications of genes encoding NF-κB transcription factors.

The NF-κB Family and Targets

NF-κB, discovered in the laboratory of David Baltimore in 1986, plays an important role in regulating inflammatory and autoimmune responses, cell proliferation, and apoptosis by regulating the expression of genes encoding inflammatory cytokines, cell adhesion molecules, and cyclooxygenase-2 (COX-2). NF-κB is known to be activated by over 450 different activators, including oxidative stress, mitogens, bacteria, and mediators of apoptosis.

Five members of the NF-κB family have been identified: NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and c-Rel that associate to form various homodimeric or heterodimeric combinations. They share a highly conserved Rel homology domain (RHD), which is responsible for DNA binding, dimerization, and interaction with IκBs. The p50/RelA (p65) heterodimer is the major NF-κB complex in most cells. The activity of NF-κB is tightly regulated by its interaction with inhibitory IκB proteins. In most resting cells, NF-κB is sequestered in the cytoplasm in an inactive form associated with inhibitory molecules such as IκBα, IκBβ, IκBε, p105, and p100. This interaction blocks the ability of NF-κB to translocate to the nucleus and bind to DNA. Following exposure to pro-inflammatory cytokines, UV light, reactive oxygen species, or bacterial and viral toxins, the NF-κB signaling cascade is activated, leading to the proteasomal degradation of IκB. This allows the translocation of unmasked NF-κB from the cytoplasm to the nucleus where it binds to NF-κB response elements in target genes and regulates their transcription. Among the NF-κB regulated genes involved in managing the response to inflammation are those encoding for iNOS, COX-2, TNFα, IL-1, IL-6, IL-8, and others. Some of these molecules have also been linked to carcinogenesis. Studies have shown that NF-κB is constitutively active in many types of human tumors.

Toll-like Receptors (TLR) signaling via NF-κB

TLRs are transmembrane proteins, expressed on various immune cells, contain an N-terminal extracellular and a C-terminal cytoplasmic domain. The N-terminal region contains leucine-rich repeats that recognize specific pathogen components. Although the ligand recognition mechanisms of the TLRs vary significantly, all TLR dimers exhibit similar overall arrangement. At least 13 different members of TLR family have been identified that detect different pathogen associated molecular patterns (PAMPs), including lipopolysaccharides, flagellin, bacterial CpG DNA, and viral RNA and DNA. Recognition of PAMPs by TLRs is considered as a key process for the induction of an inflammatory response. Following infection with a pathogen, TLRs recruit a set of Toll/Interleukin-1 receptor (TIR) domain containing adaptor proteins, such as the Myeloid Differentiation primary response 88 (MyD88), MyD88-adaptor-like (MAL), and TIR-domain-containing adapter-inducing interferon-β (TRIF), which trigger a signaling cascade leading to NF-κB activation. The organization of TLRs and interactions with adaptor proteins is complex and is beyond the scope of this short article. MyD88 that contains a TIR domain recruits TRAF and IL-1 receptor-associated kinase (IRAK) family of protein kinases that activate TAK1. In addition to the MyD88-dependent pathway, NF-κB can also be activated via the TRIF-dependent pathway, which is primarily used by TLR3 and TLR4. TRIF can directly bind to the N-terminal region of TRAF6, which leads to TAK1 activation. The activated TAK1 complex then phosphorylates and activates the IKK complex consisting of IKKα, IKKβ, and IKKγ (NEMO).

Activation of NF-κB

The activation of NF-κB occurs either via the classical pathway, which is triggered by bacterial and viral infections and pro-inflammatory cytokines, or by the alternate pathway, which is activated by members of the Tumor Necrosis Factor (TNF) family. These two pathways switch on different sets of genes and therefore mediate different immune functions. The activation of NF-κB by extracellular inducers depends on the phosphorylation and subsequent degradation of IκB proteins. Activation of NF-κB is achieved through the action of a family of serine/threonine kinases known as IκB kinase (IKK). IKK contains two catalytic subunits (IKKα and IKKβ) and a regulatory/adapter protein IKKγ (also known as NEMO). IKK activity and NF-κB activation are largely dependent on the integrity of NEMO and IKKβ. Cells devoid of IKKα can still show normal induction of NF-κB-DNA binding in response to stimuli. IKKα and IKKβ phosphorylate

IκB proteins on conserved N-terminal serine residues, known as destruction box serine residues (DSGXXS). These phosphorylation events lead to the immediate K-48 induced polyubiquitination of IκB proteins and their rapid degradation by the 26S proteasome complex. IKKα and IKKβ share about 50% sequence homology and can interchangeably phosphorylate $Ser^{32/36}$ of IκBα and $Ser^{19/23}$ of IκBβ.

In the nucleus, recruitment of NF-κB to its target genes and regulation of NF-κB -mediated transcriptional activation are mediated mainly by phosphorylation and acetylation of NF-κB, which enhance its DNA binding activity. Several protein kinases, including PKA, PKCζ and casein kinase II directly phosphorylate p65 (at Ser^{276}, Ser^{311}, and Ser^{529}, respectively). However, others like PI 3-K/Akt and NIK (NF-κB -inducing kinase) phosphorylate IKK, which in turn phosphorylates p65 at Ser^{536}. Reversible acetylation of NF-κB can also determine its active or inactive state. p300 and CBP acetyltransferases play a major role in the acetylation of p65, principally targeting $Lys^{218, 221, 310}$ for modification. Acetylated NF-κB is active and is resistant to the inhibitory effects of IκB. Deacetylation of p65 by histone deacetylase 3 (HDAC3) promotes its binding to IκB and leads to rapid export of deacetylated NF-κB from the nucleus into the cytoplasm. One of the target genes activated by NF-κB is that encoding IκBα. Newly synthesized IκBα can enter the nucleus, remove NF-κB from DNA, and export the complex back to the cytoplasm to restore its original latent state. Even though IκBα:p65:p50 complex constantly shuttles between the nucleus and the cytoplasm, the masking of nuclear localization signaling on p65 by IκBs result in a steady-state cytoplasmic localization of NF-κB dimers.

Interaction with other pathways:

NF-κB and its activating kinases perform an extensive cross-talk with several other upstream and downstream signaling pathways. Many intermediates that play an essential role in IKK activation are also shared with other pathways. Both IKKα and IKKβ are reported to mediate cross talk with p53, MAP kinases, and other signaling pathways. IKKα is able to target both nuclear as well as the cytosolic proteins. It can modulate gene expression through recruitment and modification of HDAC3. In addition to phosphorylating IκB, IKKα can also phosphorylate p65 and trigger its turnover and removal that can terminate NF-κB response and limit inflammation. IKKα can also directly phosphorylate cyclin D1 at Thr^{286} and induce its degradation. IKKβ is shown to phosphorylate TSC1 to inhibit its function and cause mTOR activation, which can then induce inflammation-mediated angiogenesis. IKKβ is also shown to phosphorylate SNAP-23 and promote assembly of the SNARE complex in mast cells to promote degranulation and anaphylactic reactions. Due to the presence of a nuclear-localization signal, phosphorylation of p53 at Ser^{362} and Ser^{366} by IKKβ can lead to its recruitment and ubiquitination by β-TrcP1 and lead to its proteasomal destruction. This p53 destruction is independent of Mdm2. The loss of p53 function has been linked to enhanced NF-κB activity, which can result in tumor survival via inhibition of apoptosis and activation of tumor survival genes.

Tumor Promotion by NF-κB

Unlike normal cells, in most cancer cells NF-κB is constitutively active and resides in the nucleus. In some cases, this is due to chronic stimulation of the IKK pathway, while in others the gene encoding IκBα may be defective. Several key processes, such as self-sufficiency in growth signals, insensitivity to growth inhibitors, evading apoptosis, unlimited replicative potential, tissue invasion, and sustained angiogenesis, are all enhanced following NF-κB activation. Hence, designing antitumor agents to block NF-κB activity may have great therapeutic value. Researchers have been exploring possibilities of interfering with NF-κB activation and its binding to DNA. They include inhibition of NF-κB dimerization, inhibition of NF-κB activation, use of proteasome inhibitors that block IκB degradation, and inhibition of NF-κB nuclear translocation. Since IKKα and IKKβ are shown to phosphorylate proteins involved in NF-κB signaling, they are considered as important targets for the development of chemotherapeutic agents. NSAIDs and corticosteroids are shown to mediate anti-inflammatory effects via inhibition of NF-κB activation and are shown to reduce the incidence of cancer. Certain natural compounds such as curcumin, derived from turmeric, are also potential chemotherapeutic agents and act by blocking NF-κB activation.

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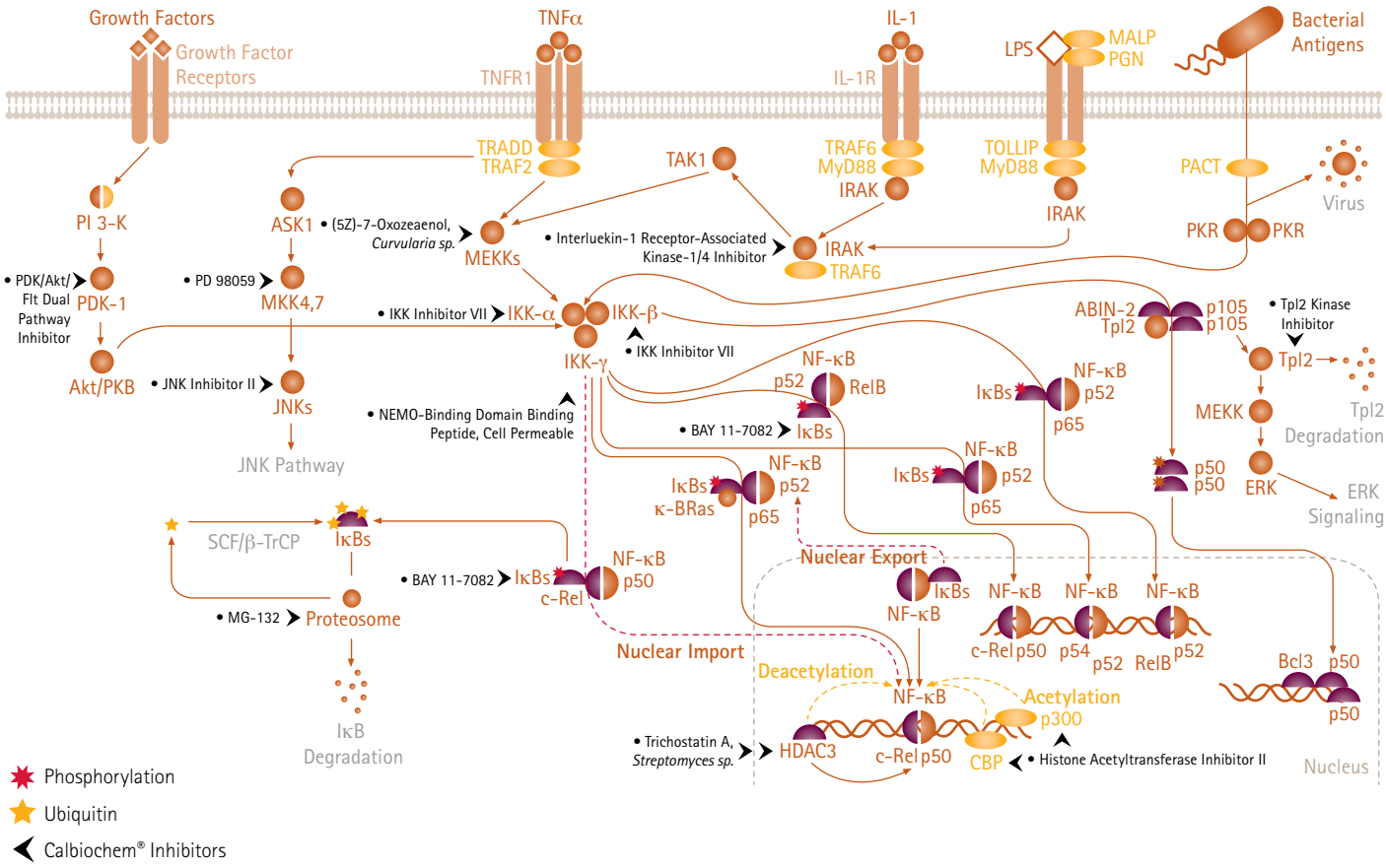
InhibitorSelect™ NF-κB Signaling Pathway Inhibitor Panel

(Catalogue No. 481487)

A panel containing 14 potent and selective inhibitors* useful for the study of NF-κB signaling pathway.

* Each inhibitor in the panel can also be purchased individually.



The Inhibitor panel includes:

Description	Qty/Pk	Catalogue No.
BAY 11-7082	1 mg	196870
IKK Inhibitor VII	1 mg	401486
Interleukin 1- Receptor-Associated-Kinase-1/4 Inhibitor	5 mg	407601
Histone Acetyltransferase Inhibitor II	10 mg	382110
MG-132	5 mg	474790
NEMO-Binding Domain-Binding Peptide, Cell-Permeable	500 µg	480025
NF-κB SN50, Cell-Permeable Inhibitor Peptide	500 µg	481480
PD 98059	5 mg	513000
PDK1/Akt/Flt Dual Pathway Inhibitor	5 mg	521275
Trichostatin A, *Streptomyces sp.*	1 mg	647925
TIRAP Inhibitor Peptide, Cell-Permeable	1 mg	613570
Tpl2 Kinase Inhibitor	1 mg	616373
(5Z)-7-Oxozeaenol, *Curvularia sp.*	1 mg	499610
JNK inhibitor II	5 mg	420119
Anhydrous DMSO	15 mL	

FEATURED NF-κB ACTIVATION INHIBITORS

Description	Comments	Qty/Pk	Catalogue No.
Andrographolide	An irreversible blocker of NF-κB and AP-1 (IC_{50} ≤ 15 µM) activation with anti-inflammatory, anti-apoptotic properties.	50 mg	172060
Aurothiomalate	Blocks NF-κB activation and PKCι-dependent Rac1 signaling. Modifies metal sensitive cysteine residues of IKK and PKCι, and inhibits the activity of IKK (IC_{50} = 10.9 µM).	50 mg	189401
Bay 11-7082	Selective, irreversible inhibitor of TNFα-inducible phosphorylation of IκB-α (IC_{50} = 10 µM) without affecting the constitutive IκB-α phosphorylation. Reduces the nuclear translocation of NF-κB.	10 mg	196870
InSolution™ BAY 11-7082	A 100 mM (10 mg/483 µL) solution of BAY 11-7082 (Catalogue No. 196870) in DMSO.	10 mg	196871
Bay 11-7085	Exhibits biological properties similar to that of BAY 11-7082 (Catalogue No. 196870). BAY 11-7085 has also been shown to have potent anti-inflammatory properties *in vivo*.	10 mg	196872
CAPE (Caffeic Acid Phenethyl Ester, Synthetic)	A cell-permeable active component of propolis from honeybee hives with antiviral, anti-inflammatory, and immunomodulatory properties. Has been shown to act as a potent and specific inhibitor of NF-κB activation.	25 mg	211200
(E)-Capsaicin	An active constituent of cayenne pepper with excitatory and desensitizing effects on a subset of primary afferent sensory neurons. Inhibits NF-κB activation by TNF.	100 mg	211275
EF24 (3,5-bis-(2-Fluorobenzylidene)-piperidin-4-one)	A cell-permeable analog of Curcumin that acts as a potent inhibitor of IKKβ inhibitor (IC_{50} = 1.9 µM) and is shown to block and NF-κB nuclear translocation (IC_{50} = 1.3 µM).	10 mg	324510
Gliotoxin, *Gladiocladium fimbriatum*	An immunosuppressive secondary metabolite produced by several pathogenic fungi. Specifically inhibits NF-κB in B and T cells in nanomolar concentrations.	1 mg	371715
Hypoestoxide, *Hypoestes rosea*	A naturally occurring, cell-permeable diterpene that acts as a selective and direct inhibitor of IκB kinase (IC_{50} = 24 µM) in TNF-α stimulated HeLa cells thereby prevents NF-κB activation.	1 mg	401006
IκB Kinase Inactive Control Peptide, Cell-Permeable (Ac-AVALLPAVLLALLAPDDRHDAGLDAMKDE-NH_2)	An inactive control for IκB Kinase Inhibitor Peptide (Catalogue No. 401477). A 14-amino acid peptide corresponding to the mutated recognition sequence of IκB (Ser^{32} → Ala and Ser^{36} → Ala), linked to the hydrophobic region of the fibroblast growth factor signal peptide.	1 mg	401478
IκB Kinase Inhibitor Peptide, Cell-Permeable (Ac-AAVALLPAVLLALLAPDDRHDSGLDSMKDE-NH2)	A 14-amino acid peptide corresponding to the active IκB phosphorylation recognition sequence, linked to the hydrophobic region of the fibroblast growth factor signal peptide to aid in cellular delivery. Specifically inhibits LPS-induced IκB degradation by IκB kinases (IKK) in RAW 264.7 cells (<50 µg/mL) and prevents NF-κB activation.	1 mg	401477
IKK Inhibitor II, Wedelolactone	A cell-permeable, selective, and irreversible inhibitor of IKKα and IKKβ (IC_{50} < 10 µM). Inhibits NF-κB -mediated gene transcription in cells by blocking the phosphorylation and degradation of IκBα.	1 mg	401474
IKK Inhibitor III, BMS-345541 (4-(2′-Aminoethyl)amino-1,8-dimethylimidazo[1,2-a] quinoxaline)	A cell-permeable, potent, selective, and allosteric site-binding inhibitor of IKK-2 (IC_{50} ~300 nM) that displays anti-inflammatory properties. Exhibits ~10-fold greater selectivity for IKK-2 over IKK-1 (IC_{50} ~4.0 µM).	1 mg	401480
IKK Inhibitor VII	A cell-permeable benzamido-pyrimidine compound that acts as a potent, selective, and ATP-competitive inhibitor of IKK (IC_{50} = 40 nM, 70 nM, and 200 nM for IKK-2, IKK complex, and IKK-1, respectively).	1 mg	401486
IKK Inhibitor X (N-(6-Chloro-9H-β-carbolin-8-yl)nicotinamide)	A cell-permeable β-carboline that acts as a potent, ATP-competitive, and reversible inhibitor of IKK (IC_{50} = 88 nM) with selectivity over 14 other commonly studied kinases (IC_{50} > 100 µM), including NF-κB inducing kinase.	5 mg	401489
IKK Inhibitor XII	A cell-permeable amino-diarylbenzamide compound that acts as a potent ATP site-targeting inhibitor against IKK-1 and IKK-2 (pIC_{50} = 6.4 and 7.0, respectively).	5 mg	401491
IKK Inhibitor XIII, 17-AJB	A cell-permeable, irreversible inhibitor against IKK-β activity (IC_{50} = 300 nM).	1 mg	401493
IKK-2 Inhibitor IV ([5-(p-Fluorophenyl)-2-ureido]thiophene-3-carboxamide, TPCA-1)	A cell-permeable, reversible, ATP-competitive, and potent inhibitor of IKK-2 (IC_{50} = 18 nM) that exhibits selectivity over IKK-1, JNK, and p38 MAPK.	500 µg	401481
InSolution™ IKK-2 Inhibitor IV	A 10 mM (500 µg/179 µL) solution of IKK-2 Inhibitor IV (Catalogue No. 401481) in DMSO.	500 µg	401484

FEATURED NF-κB ACTIVATION INHIBITORS

Description	Comments	Qty/Pk	Catalogue No.
IKK-2 Inhibitor V (N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide, IMD-0354)	A cell-permeable IKK-2 inhibitor that selectively blocks IκBα phosphorylation (IC_{50} ~250 nM) and prevents the induction of NF-κB p65 nuclear translocation.	5 mg	401482
IKK-2 Inhibitor VI (5-Phenyl-2-ureido)thiophene-3-carboxamide)	An ureido-thiophenecarboxamide compound that acts as a potent, cell-permeable, reversible, and ATP-competitive inhibitor of IKK-2 (IC_{50} = 13 nM).	1 mg	401483
IKK-2 Inhibitor VIII (2-Amino-6-(2-(cyclopropylmethoxy)-6-hydroxyphenyl)-4-(4-piperidinyl)-3-pyridinecarbonitrile, ACHP)	A cell-permeable piperidinyl-pyridine compound that acts as a selective inhibitor of IKK-2 (IC_{50} = 8.5 and 250 nM for IKK-2 and IKK-1, respectively).	1 mg	401487
IKK-2 Inhibitor XI (5-Phenyl-3-ureido)-thiophene-2-carboxamide	A cell-permeable thiophenecarboxamide-ureido compound that acts as a potent, ATP-binding pocket-targeting inhibitor of IKK-2 (IC_{50} = 25 nM).	5 mg	401490
IKK-2 Inhibitor, SC-514	A cell-permeable, potent, reversible, ATP-competitive and highly selective inhibitor of IKK-2 (IC_{50} ~3-12 µM for IKK-2 homodimer, IKK-1/IKK-2 heterodimer, and IKK-2).	1 mg	401479
InSolution™ IKK-2 Inhibitor, SC-514	A 25 mM (1 mg/178 µL) solution of IKK-2 Inhibitor, SC-514 (Catalogue No. 401479) in DMSO.	1 mg	401485
IKK-3 Inhibitor IX (5-(5,6-Dimethoxybenzimidazol-1-yl)-3-(2-methanesulfonyl-benzyloxy)-thiophene-2-carbonitrile	A thiophenecarbonitrile compound that acts as a potent ATP-competitive inhibitor of IKK-3 (IKK-ε, IC_{50} = 40 nM).	5 mg	401488
Isohelenin, *Inula sp.*	A cell-permeable sesquiterpene lactone that acts as a highly specific, potent, and irreversible inhibitor of NF-κB activation by preventing IκBα degradation. Does not affect the DNA binding activity of activated NF-κB.	1 mg	416157
NEMO-Binding Domain Binding Peptide, Cell-Permeable (DRQIKIWFQNRRMKWKKTALDWSWLQTE)	A cell-permeable Antennapedia-NBD (wild type) fusion peptide that blocks the association of NEMO with the IKK complex and prevents NF-κB activation.	500 µg	480025
NBD-Binding Peptide II, Cell Permeable, CTP-NBD (CTP; YGRRARRRARR; NBD; TALDWSWLQTE)	A cell-permeable cytoplasmic transduction peptide (CTP) fused to the NEMO binding domain (NBD). Inhibits TNFα-stimulated NF-κB activity (IC_{50} ~ 50 µM in HCT116 cells). Interferes with the formation of active IKK complex and thereby prevents IκBα phosphorylation and its subsequent proteasomal degradation.	5 mg	480026
NEMO-Binding Domain Binding Peptide, Cell-Permeable, Negative Control (DRQIKIWFQNRRMKWKK-TALDASALQTE)	A cell-permeable, Antennapedia-NBD mutated (Trp739 → Ala and Trp741 → Ala) fusion peptide analog of NEMO-Binding Domain Binding peptide (Catalogue No. 480025) that serves as a negative control.	500 µg	480030
NEMO NOA Pepotide, Cell-Permeable, A-UBI (NEMO Ubiquitin Binding Domain Inhibitor)	A cell-permeable 37-mer peptide derived from the NEMO NOA ubiquitin binding site that attenuates NF-κB activation via blocking interactions between K63-polyubiquitin chains and the NOA ubiquitin binding domain of NEMO (K_d = 17 µM for CC2-LZ/A-UBI complex).	2 mg	481418
NF-κB Activation Inhibitor 6-Amino-4-(4-phenoxyphenylethylamino)quinazoline	A cell-permeable quinazoline compound that acts as a potent inhibitor of NF-κB transcriptional activation (IC_{50} = 11 nM in Jurkat cells).	1 mg	481406
InSolution™ NF-κB Activation Inhibitor	A 10 mM (1 mg/281 µL) solution of NF-κB Activation Inhibitor (Catalogue No. 481406) in DMSO.	1 mg	481407
NF-κB Activation Inhibitor II, JSH-23 (4-Methyl-N^1-(3-phenylpropyl)benzene-1,2-diamine)	A cell-permeable, selective blocker of the nuclear translocation of NF-κB p65 that does not affect IκB degradation (IC_{50} = 7.1 µM in LPS-stimulated macrophages RAW 264.7 stably transfected with NF-κB-SEAP-NPT). Shown to suppress DNA-binding of NF-κB.	5 mg	481408
NF-κB Activation Inhibitor III (3-Chloro-4-nitro-N-(5-nitro-2-thiazolyl)-benzamide, SM-7368)	A cell-permeable inhibitor of TNF-α-stimulated NF-κB activation and subsequent upregulation of MMP-9 in HT1080 cells (complete inhibition at 10 µM).	5 mg	481411
NF-κB Activation Inhibitor IV ((E)-2-Fluoro-4′-methoxystilbene)	A cell-permeable trans-stilbene Resveratrol (Catalogue No. 554325) analog that is shown to be ~130-fold more potent than Resveratrol in inhibiting TNF-α-stimulated NF-κB reporter activity in 293T cells (IC_{50} = 150 nM).	10 mg	481412

FEATURED NF-κB ACTIVATION INHIBITORS

Description	Comments	Qty/Pk	Catalogue No.
NF-κB Activation Inhibitor V, 5HPP-33 (5-Hydroxy-(2,6-diisopropylphenyl)-1H-isoindole-1,3-dione)	A cell-permeable N-phenylpthalimide compound that inhibits IL-1-induced NF-κB translocation in HeLa cells (IC_{50} = 530 nM). Effectively suppresses the proliferation of various cancer cells, including several Paclitaxel-resistant lines.	25 mg	100066
NF-κB Activation Inhibitor VI, BOT-64 (6,6-Dimethyl-2-(phenylimino)-6,7-dihydro-5H-benzo[1,3] oxathiol-4-one)	A cell-permeable benzoxathiole compound that acts as an IKK-2 inhibitor, presumably by targeting the Ser^{177} and/or Ser^{181} residues in the kinase's activation loop domain.	5 mg	481414
NF-κB Activation Inhibitor VII, CID-2858522 (1-(3,5-di-tert-butyl-4-hydroxyphenyl)-2-(2-(3-hydroxypropylamino)-5,6-dimethyl-1H-benzo[d]imidazol-1-yl) ethanone;)	A cell-permeable, selective inhibitor of PMA-induced NF-κB activation PKC pathway activators such as PMA (IC_{50} = 70 nM).	10 mg	480457
NF-κB Activation Inhibitor VIII, EVP4593 (N4-(4-phenoxyphenethyl)quinazoline-4,6-diamine, SOC Inhibitor)	A cell-permeable quinazoline derivative that blocks PMA/PHA-induced NF-κB activation (IC_{50} = 11 nM).	5 mg	481417
NF-κB SN50, Cell-Permeable Inhibitor Peptide (AAVALLPAVLLALLAPVQRKRQKLMP)	Contains the nuclear localization sequence (NLS) of the transcription factor NF-κB p50 linked to the hydrophobic region (h-region) of the signal peptide of Kaposi fibroblast growth factor (K-FGF). The peptide N-terminal K-FGF h-region confers cell-permeability, while the NLS (360-369) inhibits translocation of the NF-κB into the nucleus.	500 µg 1 mg	481480
NF-κB SN50M, Cell-Permeable Inactive Control Peptide (AAVALLPAVLLALLAPVQRNGQKLMP)	An inactive control for SN50 peptide (Catalogue No. 481480). Corresponds to the SN50 peptide sequence with substitutions of Lys^{363} for Asn and Arg^{364} for Gly in the NLS region.	500 µg	481486
Oridonin, *R. rubescens*	A cell-permeable inhibitor of NF-κB activity that displays anti-proliferative (ED_{50} ~ 2.7 µg/mL in lymphoid malignant cells) and anti-angiogenic properties. Inhibits p65 NF-κB transcriptional activity by disrupting NF-κB DNA-binding activity without interfering with its nuclear translocation	5 mg	496915
(5Z)-7-Oxozeaenol, *Curvularia sp.*	A cell-permeable fungal resorcylic lactone that acts as a selective, highly potent, ATP-comeptitive, irreversible inhibitor of MAPKKK TAK1 activity (IC_{50} = 8 nM).	1 mg	499610
Parthenolide, Tanacetum parthenium	A sesquiterpene lactone with anti-inflammatory, antisecretory, and spasmolytic properties that also inhibits NF-κB and activation of MAP kinase.	50 mg	512732
Pristimerin	A cell-permeable dienone-phenolic triterpenoid that blocks proteasome chymotrypsin subunit (IC_{50} = 2.2 and 3.0 µM against purified rabbit 20S and human 26S proteasome, respectively) and cellular NF-κB pathway/IKK activation.	5 mg	530070
Ro106-9920	A cell-permeable tetrazolopyridazine-phenylsulfoxide compound that acts as a highly selective, irreversible inhibitor of IκBαee ubiquitination (IC_{50} = 2.3 µM). Also reported to prevent degradation of IκBαee in MM6 cells (complete inhibition at ~3-5 µM).	1 mg 5 mg	557550
TIRAP Inhibitor Peptide, Cell-Permeable (Ant-Tirap138-151)	A synthetic, cell-permeable peptide that corresponds to mouse toll-interleukin 1 receptor (TIR) domain-containing adapter protein 138-151 (TIRAP) fused to the Drosophila antennapedia sequence. Shown to specifically inhibit LPS-induced NF-κB activation. Also reported to block IκBα degradation.	1 mg	613570
TIRAP Inhibitor Peptide, Control, Cell-Permeable (Ant-Tirap151-138)	A cell-permeable synthetic peptide containing mouse toll-interleukin 1 receptor (TIR) domain-containing adapter protein 151-138 reverse sequence (TIRAP; also called Mal (MyD88-adapter-like), fused to the Drosophila antennapedia sequence. Serves as a control for TIRAP Inhibitor Peptide (Catalogue No. 613570).	1 mg	613571
(5Z)-Zeaenol, *Curvularia sp.*	A cell-permeable resorcylic acid lactone compound that may serve as a negative control for TAK1 (TGF-β-Activated Kinase-1) inhibition studies.	1 mg	499609

ANTIBODIES FOR NF-κB RESEARCH

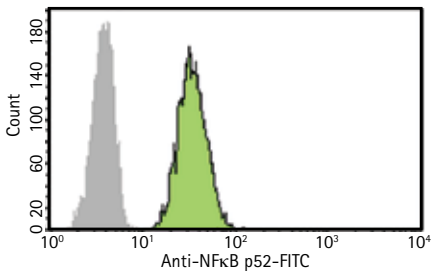
Description	Species	Applications	Qty	Catalogue No.
Anti-IκB-α Antibody	Human, Mouse	IB, IP	200 µg	06-494
Anti-IκBα Rabbit Antibody	Human, Mouse, Rat	IC, IB, IP	100 µL	400001
Anti-IκBα	Human, Mouse, Rat	ELISA, IB, IP, (IH)P	100 µL	07-1483
PhosphoDetect™ Anti-IκBα (pSer$^{32/36}$) Mouse (39A1413)	Most mammalian species	IB, IP	100 µg	OP142
Anti-IKKα, clone 14A231	Human, Monkey, Mouse	IB	100 µg	05-536
Anti-IKKα Mouse mAb (14A231)	Human, Monkey, Mouse	IB	100 µg	OP133
Anti-IKKα	Most mammalian species	IB, IP	150 µg	07-1007
Anti-IKKα, clone Y463, Rabbit Monoclonal	Human, Mouse, Rat	IB, IC, IP, FC, IH(P)	100 µL	04-365
Anti-IKKβ, Mouse Monoclonal (10AG2)	Human	IB	100 µg	OP134
Anti-IKKβ, Mouse Monoclonal (10AG2)	Human	IB	100 µg	OP134
Anti-IKKβ, clone 10AG2	Human, Mouse, Rat	IB	100 µg	05-535
Anti-IKKβ	Human, Mouse	IB, IH(P)	100 µg	07-1479
Anti-IKKβ	Most mammalian species	IB	150 µg	07-1008
Anti-IKKβ, clone Y466, Rabbit Monoclonal	Human, Mouse	FC, IB, IH(P), IP	100 µL	04-366
Anti-IKKγ	Human, Mouse	IB	100 µg	05-631
PhosphoDetect™ Anti-IKKγ/NEMO (pSer^{376}) Rabbit pAb	Human	IB	50 µL	ST1079
Anti-IKKε Antibody	Human, Mouse	IB	200 µg	07-580
Anti-phospho-IKKε (Ser^{172})	Human, Mouse, Rat	IB	100 µL	06-1340
Anti- NF-κB p50, clone E381	Human, Mouse, Rat	FC, IB, IC, IH, IH(P)	100 µL	04-234
Anti- NF-κB p50 Subunit	Human	ELISA, EMSA, IB, IP	100 µL	AB1602
Anti- NF-κB p50	Human, Mouse	IB, IP, ChiP	150 µL	06-886
Anti-NF-κB p52	Human	IB, IP	100 µL	06-413
Anti-NF-κB p65	Human, Mouse, Rat	IB, IP	100 µL	ABE136
Anti-NF-κB (p65, RelA) (Ab-2) Rabbit	Human	ELISA, EMSA, IB, IP	100 µL	PC138
Anti-NF-κB, p65 Subunit, Active Subunit, clone 12H11	Human, Mouse, Rabbit, Rat	EMSA, FC, IB, IC, IH(P)	100 µg	MAB3026
Anti-NF-κB p52	Human	EMSA, IB, IP	200 µg	05-361
Anti-NF-κB p65	Human, Mouse, Rat	IB, IP	100 µg	ABE347
Anti-NF-κB p65, C-Terminus	Human, Mouse, Rat	EMSA, IB, IP	100 µg	06-418
Anti-NF-κB, p65, clone 1G10.2	Bovine, Human, Mouse, Rat	IB, IC, IF	200 µg	05-1469
Anti-NF-κB, p65, clone E379	Human	FC, IB, IC, IH, IH(P), IP	100 µL	04-235
Anti-phospho-NF-κB (p100) (Ser^{707})	Human	DB, PIA, WB	200 µL	07-1829
Anti-NF-κB p65, Phospho-specific (Ser^{276})	Human	ELISA, IB	100 µL	AB3375
Anti-NF-κB p65, clone EP2161Y	Human	FC, IB, IC	100 µL	04-1008
Anti-Phospho-NF-κB p65 (Ser^{536}), clone EP2294Y	Human	IB, IP	100 µL	04-1000
Anti-Phospho-NF-κB (p100) (Ser^{707})	Human	DB, IB, PIA	200 µL	07-1829
Anti-NIK, C-terminus	Human	IB	100 µg	AB16528
Anti-RelB	Human	IB, IC, IF	200 µL	07-1332
Anti-RelB	Human, Mouse, Rat, Rhesus Monkey	IB, IH(P)	100 µg	06-1105
Anti-RelB (N-Term), clone EP613Y	Human	IB, IC, IH(P),IP	100 µL	04-1077
Anti-c-Rel (NF-κB)	Human	ELISA, EMSA, IB	100 µg	09-040
Anti-TBK1, clone AOW9	Canine, Chimpanzee, Human, Mouse	IB, IP	100 µL	04-856
Anti-TBK1/NAK (N-term), clone EP611Y	Human, Mouse, Rat	ICC, IB, IH(P),IP	100 µL	04-387
Anti-TBK1 Mouse (108A429)	Human	IB	100 µg	AM76
Anti-Phospho-TBK1 (Ser^{172})	Human, Mouse, Rat	IB	100 µL	07-2192

Applications:
ChIP: Chromatin Immunoprecipitation
DB: Dot Blotting
EMSA: Electrophoretic Mobility Supershift Assay
ELISA: Enzyme-linked Immunosorbent Assay
FC: Flow Cytometry
IB: Immunoblotting
IC: Immunocytochemistry
IH: Immunohistochemistry
IP: Immunoprecipitation
PIA: Peptide Inhibition Assay

Milli-Mark® Anti-NF-κB p52-FITC

(Qty: 100 tests, Catalogue No. FCMAB346F)

Purified mouse monoclonal IgG_{2a} conjugated to FITC. Supplied in PBS with 0.1% sodium azide and 15 mg/mL BSA.
Reactivity: Human. Does not cross-react with mouse or rat.
Application: Flow Cytometry

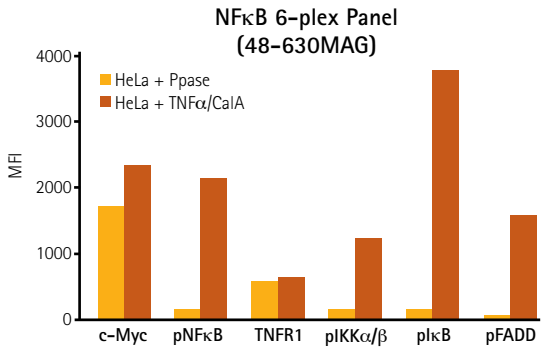


Flow cytometric analysis of HeLa cells stained with Anti-NFκB p52-FITC conjugated. Cells were stained with the antibody (green) or with an isotype control (gray).

MILLIPLEX® MAP NF-κB Magnetic Bead 6-plex Panel

(Catalogue No. 48-630)

- Save time and Save sample
- Simultaneous multianalyte detection based on Luminex® XMAP® technology.
- Consistent Results
- Easy to use



Description	Catalogue No.
MILLIPLEX® MAP 6-plex NF-κB, Magnetic Beads (20X)	42-630MAG
MILLIPLEX® 6-plex NF-κB, Biotin (20X) (Detection Antibody)	44-630KMG
MILLIPLEX® MAP Lysis Buffer (1X)	43-040
MILLIPLEX® MAP Assay Buffer 1 (1X)	43-010
MILLIPLEX® MAP HeLa Cell Lysate: Lambda Phosphatase	47-229
MILLIPLEX® MAP A431 Cell Lysate: EGF	47-210
MILLIPLEX® MAP HeLa Cell Lysate: TNFα/Calyculin A	47-230
MILLIPLEX® MAP Streptavidin-Phycoerythrin (25X)	45-001H
MILLIPLEX® MAP Amplification Buffer (1X)	43-024A
Set of one 96-well Filter Plate and 2 sealers	
Set of one 96-well Plate and 2 sealers	
3 Empty Mixing Bottles	

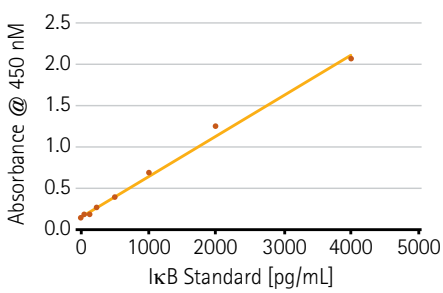
IκB STAR ELISA Kit

(Qty: 96 Assays, Catalogue No. 17-485)

- The STAR (Signal Transduction Assay Reaction) ELISA kit is a solid phase sandwich ELISA assay.
- Fast and sensitive
- Can be used with whole cell extracts
- Sensitivity: 62.5 pg/mL
- Detection Range: 62.5 to 4000 pg/mL
- Species Reactivity: Human, mouse and rat

Components included:

- Capture Plate pre-coated with anti-IκB antibody: (Part No. 17-485A)
- Anti- IκB detection antibody: (Part No. 17-485B)
- Ready to use ELISA Diluent: (Part No. 17-485C)
- 25X ELISA Wash Buffer: (Part No. 17-485D)
- Anti-Rabbit IgG HRP conjugate: (Part No. 17-485E)
- HRP Diluent: (Part No. 17-485F)
- TMB Solution: (Part No. 17-485G)
- Stop Solution: (Part No. 17-485H)
- IκB Standard: (Part No. 17-485I
- Plate Covers: Two plate covers.
- Instruction manual



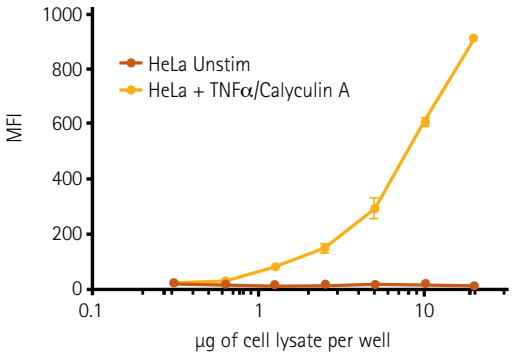
Typical IκB Standard Curve 100 µL of progressive 2-fold dilutions of the IκB standard included in the kit

MILLIPLEX® MAP Phospho NF-κB (Ser^{536}) MAPmate™

(Qty: 1 kit, Catalogue No. 46-702)

- For detection of phosphorylated NF-κB (Ser^{536}) in cell lysates using Luminex® 100™ IS, 200™, or HTS system.
- A rapid, convenient alternative to Western blotting and immunoprecipitation.
- **Negative Control**: The MILLIPLEX MAP HeLa Cell lysate (unstimulated).
- **Positive Control**: HeLa cell lysate (TNFα + calyculin A treated)
- Each kit contains sufficient reagents for 100 individual assays.
- MAPmate™ is compatible with both Assay Buffer 1 (Catalogue No. 43-010) and Assay Buffer 2 (Catalogue No.43-041)

Detection of Phospho NFκB in HeLa Cells



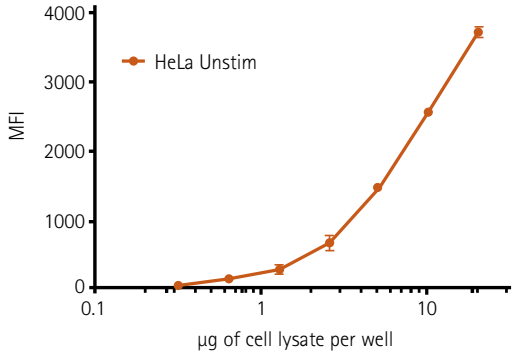
Description	Luminex® Bead No.	Volume	Qty	Catalogue No.
MILLIPLEX® MAP Anti NF-κB Beads (20X)	85	140 µL	1 tube	42-702
MILLIPLEX® MAP Anti-Phospho NF-κB (Ser^{536}), biotin (20X)	-	140 µL	1 tube	44-702
MILLIPLEX® MAP HeLa Cell Lysate, unstimulated	-	-	1 vial	47-205
MILLIPLEX® MAP HeLa Cell Lysate, TNFα+calyculin A	-	-	1 vial	47-230

MILLIPLEX® MAP Total NF-κB MAPmate™

(Qty: 1 kit, Catalogue No. 46-701)

- For detection of Total NF-κB in cell lysates using the Luminex® 100™ IS, 200™, or HTS system.
- A rapid, convenient alternative to Western blotting and immunoprecipitation.
- The MILLIPLEX MAP HeLa cell lysate (unstimulated) may be utilized as a positive control.
- Each kit contains sufficient reagents for 100 individual assays.

Detection of Total NFκB in HeLa Cells



Description	Luminex® Bead No.	Volume	Qty	Catalogue No.
MILLIPLEX® MAP Anti NF-κB Beads (20X)	85	140 µL	1 tube	42-701
MILLIPLEX® MAP Anti-Total NF-κB, biotin (20X)	-	140 µL	1 tube	44-701

NF-κB p50/p65 EZ-TFA Transcription Factor Assays

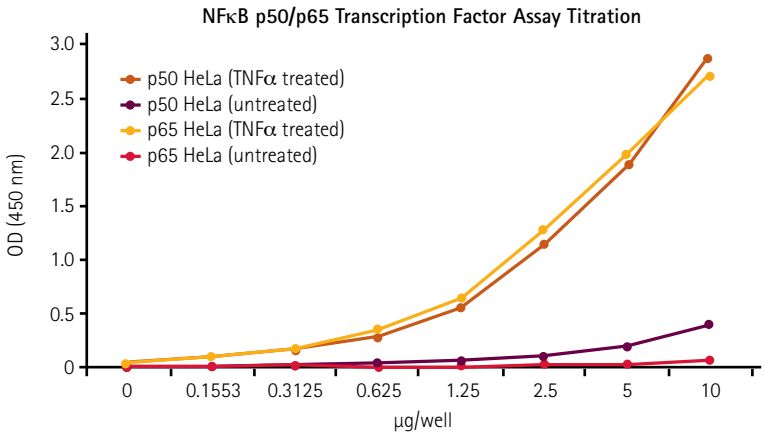
For Chemiluminescent Detection

Description	Catalogue No.
NF-κB p50/p65	70-610
NF-κB p50	70-615
NF-κB p65	70-620

For Colorimetric Detection:

Description	Catalogue No.
NF-κB p50/p65	70-510
NF-κB p50	70-515
NF-κB p65	70-520

- The Non-radioactive chemiluminescent NF-κB p50/p65 Transcription Factor Assay kit.
- 96-well format
- Suitable for use with human, mouse, and rat samples.
- Utilizes a probe composed of a double stranded biotinylated oligonucleotide containing the flanked DNA binding consensus sequence for NF-κB (5'-GGGACTTTCC-3').
- The active NF-κB from nuclear extract is immobilized on capture probe bound to the streptavidin plate well.
- The bound p50 and/or p65 subunits are detected with specific primary antibodies, Rabbit anti-NF-κB p50 and Rabbit anti-NF-κB p65.
- A highly sensitive HRP-conjugated secondary antibody is then used for detection.



Titration of TNFα-treated HeLa cell (0.2 µg/mL for 30 min. at 37 °C) nuclear extract and untreated HeLa nuclear extracts. The assay was performed using the 70-510 kit and its various components with normal parameters of the assay as outlined in the assay protocol.

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