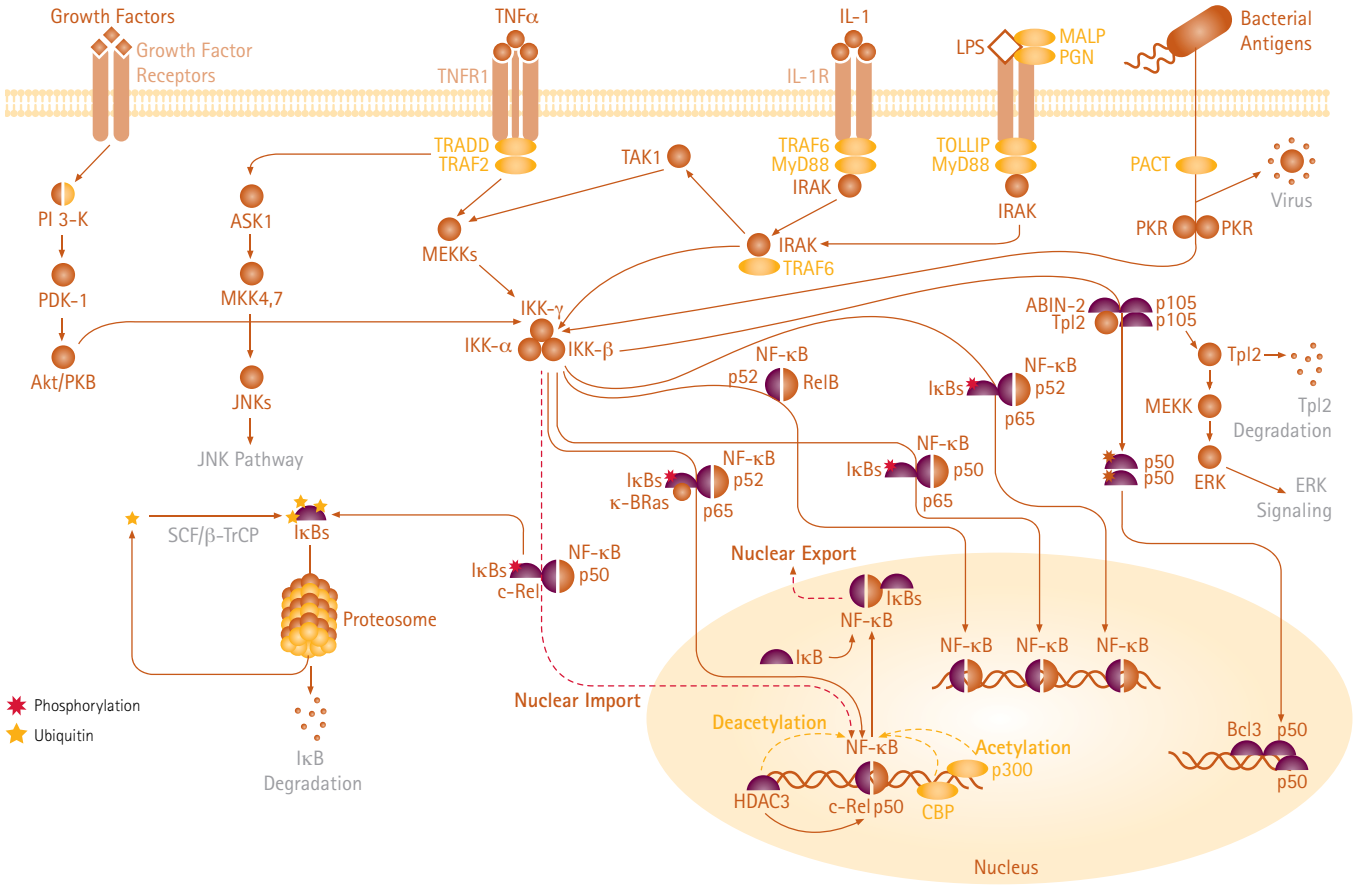
Pathways and Biomarkers of

Toll-like Receptor (TLR) Signaling



Toll-like Receptors (TLRs) are transmembrane proteins that are expressed on various immune cells. The extracellular N-terminal region of TLRs recognizes specific pathogen components. 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.

The Toll-like receptors also work in conjunction with the complement system to provide key innate defenses. Their interplay reinforces innate immunity and regulates excessive inflammation, through synergistic as well as antagonistic interactions. Complement can inhibit TLR-induced production of IL-12. Also, it has been reported that activation of C5aR in macrophages can inhibit TLR4- induced mRNA expression of IL-12p35, IL-12/IL-23p40, IL-23p19 and IL-27p28, and production of IL-12, IL-23 and IL-27 proteins. Here the underlying crosstalk mechanism is believed to involve the induction of PI-3 kinase and ERK1/2 signaling that suppresses key transcription factors, IRF-1 and IRF-8 that regulate the expression of the IL-12 family of cytokines.

Toll-like receptors also cooperate with inflammasome and the outcome of their interaction leads to IL-1β and IL-18 secretion. This is achieved by two stepwise processes; the transcriptional induction of pro-IL-1β and pro-IL-18 by TLR or nucleotide binding oligomerization domain-containing protein (NOD) signaling, followed by their proteolytic processing via inflammasome action. The first process is elicited by TLR ligands or by TNF-α, while the second step signal can be provided by pore-forming toxins, amyloid peptides, uric acid crystals and K^+ efflux.

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Tools for the study of Inflammation: TLRs and NF-κB

Description	Cat. No.
Antibodies	
Anti-CD282 (TLR2) (human) Antibody, PE, clone TL2.1	MABF349
Anti-CD284 (TLR4) (human) Antibody, PE, clone HTA125	MABF350
Anti-c-Rel (NF- B) Antibody	09-040
Anti-HDAC3 Antibody, clone 3G6	05-813
Anti-IκBα Antibody	07-1483
Anti-IKKα Antibody, clone 14A231	05-536
Anti-IKKβ Antibody, clone 10AG2	05-535
Anti-IL-12R-β-1 Antibody	06-1089
Anti-IL18R1 Antibody	ABF303
Anti-Interleukin-18 (IL-18) Antibody	06-1115
Anti-IRAK Antibody	06-872
Anti-NF-κB p52 Antibody	06-413
Anti-NF-κB, p65 Antibody, clone 1G10.2	05-1469
Anti-NIK, C-terminus Antibody	AB16528
Anti-phospho NFκB p52, (Ser222) Antibody	ABS1515
Anti-phospho-IKKε (Ser172) Antibody	06-1340
Anti-phospho-MKK4 Antibody (Ser257/Thr261)	ABS160
Anti-Phospho-TBK1 (Ser172) Antibody	07-2192
Anti-RelB Antibody	07-1332
Anti-TLR2 Antibody, clone 19B6.2	MABF84
Anti-TRAF6 Antibody	06-1110
Assays and Kits	
IκB STAR ELISA Kit	17-485
MILLIPLEX® MAP NF?B Magnetic Bead Kit 6-plex	48-630MAG
MILLIPLEX® MAP Phospho NF-κB (Ser536) Magnetic Bead MAPmate™	46-702MAG
MILLIPLEX® MAP Total NF-κB Magnetic Bead MAPmate™	46-701MAG
Small Molecules	
(5Z)-7-Oxozeaenol, *Curvularia sp.*	499610
BAY 11-7082	196870
Histone Acetyltransferase Inhibitor II	382110
IKK Inhibitor VII	401486
Interleukin 1- Receptor-Associated-Kinase-1/4 Inhibitor	407601
MEK Inhibitor II	444938
NEMO-Binding Domain-Binding Peptide, Cell-Permeable	480025
NF-κB SN50, Cell-Permeable Inhibitor Peptide	481480
TIRAP Inhibitor Peptide, Cell-Permeable	613570
Tpl2 Kinase Inhibitor	616373
Trichostatin A, *Streptomyces sp.*	647925

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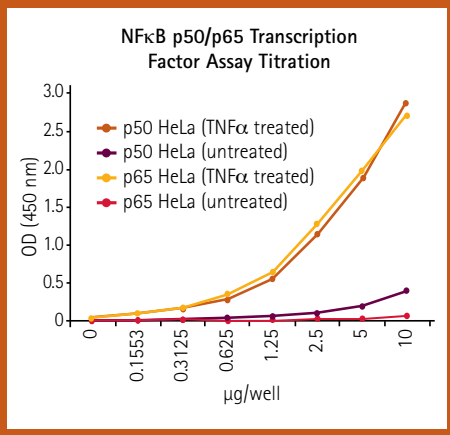
▶ TECHNOLOGY HIGHLIGHT

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

The Non-radioactive 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.

Ordering Information

For Chemiluminescent Detection	
NF-κB p50/p65	70-610
NF-κB p50	70-615
NF-κB p65	70-620
For Colorimetric Detection	
NF-κB p50/p65	70-510
NF-κB p50	70-515
NF-κB p65	70-520