Phospho-NFκB Pathway Sampler Kit

E051022

Kits Includes	Cat.	Quantity	Application	Reactivity	Source
GSK3β(Phospho-Ser9) Antibody	E011002-1	50μg/50μl	IHC, WB,IF	Human, Mouse, Rat	Rabbit
IKK α (Phospho-Thr23) Antibody	E011129-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
NFκB-p65 (Phospho-Ser276) Antibody	E011011-1	50μg/50μl	IHC, WB,IF	Human, Mouse, Rat	Rabbit
P38 MAPK(Phospho-Tyr182) Antibody	E011253-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
NFκB-p105/p50 (Phospho-Ser907) Antibody	E011019-1	50μg/50μl	IHC, WB	Human	Rabbit

GSK3B protein is a serine-threonine kinase, belonging to the glycogen synthase kinase subfamily. It is involved in energy metabolism, neuronal cell development, and body pattern formation. Polymorphisms in this gene have been implicated in modifying risk of Parkinson disease, and studies in mice show that overexpression of this gene may be relevant to the pathogenesis of Alzheimer disease. Alternatively spliced transcript variants encoding different isoforms have been found for this gene. Participates in the Wnt signaling pathway. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN. Phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA. Phosphorylates MUC1 in breast cancer cells, and decreases the interaction of MUC1 with CTNNB1/beta-catenin. Phosphorylates CTNNB1/beta-catenin.

Glycogen synthase kinase 3 (GSK-3), a serine-threonine kinase with two isoforms (alpha and beta), was originally discovered as a key enzyme in glycogen metabolism. GSK-3 was subsequently shown to function in cell division, proliferation, motility and survival. GSK-3 plays a role in a number of pathological conditions including cancer and diabetes and is increasingly seen as an important component of neurological diseases. GSK-3 phosphorylates tau and presenilin-1, which are involved in the development of Alzheimer's disease. Both isoforms of GSK-3 are ubiquitously expressed, although particularly high levels of GSK-3beta are found in the brain where it is involved in synaptic plasticity, possibly via regulation of NMDA receptor trafficking. GSK-3 phosphorylates over 40 different substrates including signaling proteins, transcription factors and structural proteins, and is part of the signal transduction cascade of a large number of growth factors and cytokines. The activity of GSK is regulated by phosphorylation (Akt, S6K, RSK, PKA and PKC), dephosphorylation (PP1 and PP2A), and by binding to protein complexes (with beta-catenin, axin, CK1 and the APC complex). IKBKE is a noncanonical I-kappa-B (see MIM 164008) kinase (**IKK**) that is essential for regulating antiviral signaling pathways. IKBKE has also been identified as a breast cancer (MIM 114480) oncogene and is amplified and overexpressed in over 30% of breast carcinomas and breast cancer

cell lines. Phosphorylates inhibitors of NF-kappa-B thus leading to the dissociation of the inhibitor/NF-kappa-B complex and ultimately the degradation of the inhibitor. May play a special role in the immune response.

NFKB1 gene encodes a 105 kD protein which can undergo cotranslational processing by the 26S proteasome to produce a 50 kD protein. The 105 kD protein is a Rel protein-specific transcription inhibitor and the 50 kD protein is a DNA binding subunit of the NF-**kappa**-B (NFKB) protein complex. NFKB is a transcription regulator that is activated by various intra- and extra-cellular stimuli such as cytokines, oxidant-free radicals, ultraviolet irradiation, and bacterial or viral products. Activated NFKB translocates into the nucleus and stimulates the expression of genes involved in a wide variety of biological functions. Inappropriate activation of NFKB has been associated with a number of inflammatory diseases while persistent inhibition of NFKB leads to inappropriate immune cell development or delayed cell growth. Two transcript variants encoding different isoforms have been found for this gene. NF-**kappa**-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-**kappa**-B is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/**p65**, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52 and the heterodimeric **p65**-p50 complex appears to be most abundant one. The dimers bind at **kappa**-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different **kappa**-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors, respectively. NF-**kappa**-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-**kappa**-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-**kappa**-B inhibitor (I-**kappa**-B) family. In a conventional activation pathway, I-**kappa**-B is phosphorylated by I-**kappa**-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-**kappa**-B complex which translocates to the nucleus. NF-**kappa**-B heterodimeric **p65**-p50 and RelB-p50 complexes are transcriptional activators. The NF-**kappa**-B p50-p50 homodimer is a transcriptional repressor, but can act as a transcriptional activator when associated with BCL3. NFKB1 appears to have dual functions such as cytoplasmic retention of attached NF-**kappa**-B proteins by p105 and generation of p50 by a cotranslational processing. The proteasome-mediated process ensures the production of both p50 and p105 and preserves their independent function, although processing of NFKB1/p105 also appears to occur post-translationally. p50 binds to the **kappa**-B consensus sequence 5'-GGRNNYYCC-3', located in the enhancer region of genes involved in immune response and acute phase reactions. In a complex with MAP3K8, NFKB1/p105 represses MAP3K8-induced MAPK signaling; active MAP3K8 is released by proteasome-dependent degradation of NFKB1/p105.

NF-kappaB (nuclear factor-kappa B) is a rapidly acting primary transcription factor found in all cell types. It is involved in cellular responses to stimuli such as cytokines and stress and plays a key role in regulating the immune response to infection. In unstimulated cells NF-kappaB dimers are sequestered inactively in the cytoplasm by a protein complex called inhibitor of kappa B (IkappaB). IkappaB inactivates NF-kappaB by masking the nuclear localization signals (NLS). Activation of NF-kappaB occurs via degradation of IkappaB, a process that is initiated by its phosphorylation by IkappaB kinase (IKK). Phosphorylated IvB becomes dissociated from NF-kappaB, unmasking the NLS. Phosphorylation also results in IkappaB ubiquitination and targeting to the proteasome. NF-kappaB can now enter the nucleus and regulate gene expression. NF-kappaB turns on expression of IkappaB forming a negative feedback loop.

MAPK14 protein is a member of the MAP kinase family. MAP kinases act as an integration point for multiple biochemical signals, and are involved in a wide variety of cellular processes such as proliferation, differentiation, transcription regulation and development. This kinase is activated by various environmental stresses and proinflammatory cytokines. The activation requires its phosphorylation by MAP kinase kinases (MKKs), or its autophosphorylation triggered by the interaction of MAP3K7IP1/TAB1 protein with this kinase. The substrates of this kinase include transcription regulator ATF2, MEF2C, and MAX, cell cycle regulator CDC25B, and tumor suppressor p53, which suggest the roles of this kinase in stress related transcription and cell cycle regulation, as well as in genotoxic stress response. Four alternatively spliced transcript variants of this gene encoding distinct isoforms have been reported. Responds to activation by environmental stress, pro-inflammatory cytokines and lipopolysaccharide (LPS) by phosphorylating a number of transcription factors, such as ELK1 and ATF2 and several downstream kinases, such as **MAPK**APK2 and **MAPK**APK5. Plays a critical role in the production of some cytokines, for example IL-6. May play a role in stabilization of EPO mRNA during hypoxic stress. Isoform Mxi2 activation is stimulated by mitogens and oxidative stress and only poorly phosphorylates ELK1 and ATF2. Isoform Exip may play a role in the early onset of apoptosis

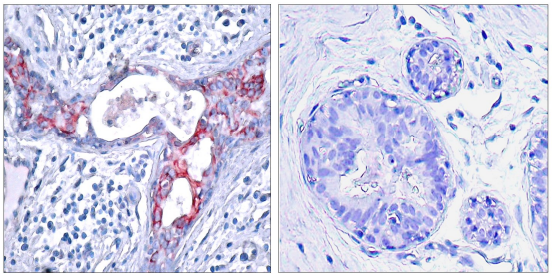
MAPKs (mitogen-activated protein kinases) are serine-threonine kinases that regulate a wide variety of cellular functions. Six groups of MAPK have so far been identified: Extracellular signal-regulated kinases (ERK1, ERK2), c-Jun N-terminal kinases (JNKs), p38 isoforms (MAPK11, MAPK12, MAPK13, MAPK14), ERK5 (MAPK7), ERK3 (MAPK6) and ERK4 (MAPK4) and ERK7/8 (MAPK15). ERK 1 and ERK 2 transduce signals from growth factors and are key in regulating differentiation and proliferation in many cell types. Upon activation by MEK, ERK1 and 2 translocate to the nucleus where they phosphorylate transcription factors such as Elk1 and downstream kinases such as p90 RSK. JNK 1,2 and 3 (sometimes known as SAPKs or stress-activated kinases) and the p38 MAPKs (alpha-, beta-, delta and gamma- isoforms) are activated by UV irradiation, inflammatory cytokines and hyperosmolarity. The p38 MAPKs are also activated by lipopolysaccharide. Dysregulation of MAPK kinase pathways has been associated with

various diseases including cancer (ERK), neurodegeneration (JNK) and inflammation (p38).

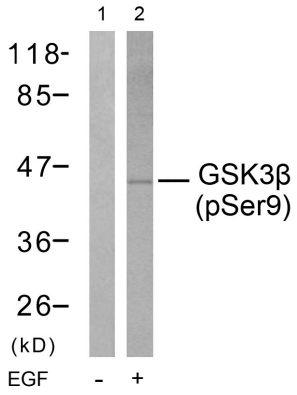
GSK3β (Phospho-Ser9) Antibody

E011002

Catalog Number: E011002-1, E011002-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human GSK3β around the phosphorylation site of serine 9 (T-T-S^P-F-A).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: GSK3β (phospho-Ser9) antibody detects endogenous levels of GSK3β only when phosphorylated at serine 9.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100 IF: 1:100-200
Swiss-Prot No. : P49841
References:
Fan G, et al. (2003) J Biol Chem. 278(52): 52432-52436.
Barry FA, et al. (2003) FEBS Lett. 553(1-2): 173-178.
Welsh, et al. (1996) Trends Cell Biol. 6: 274-279.
Srivastava A K, et al. (1998) Mol Cell Biochem. 182: 135-141.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using GSK3β (phospho-Ser9) antibody (E011002).

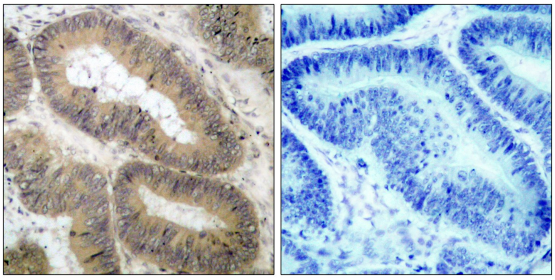


Western blot analysis of extracts from HeLa cells using GSK3β (phospho-Ser9) antibody (E011002).

IKKα (Phospho-Thr23) Antibody

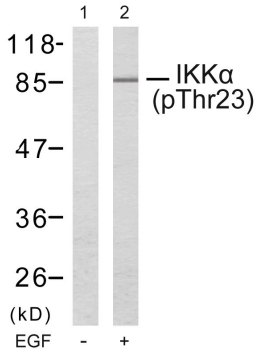
E011129

Catalog Number: E011129-1, E011129-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human IKKα around the phosphorylation site of threonine 23 (L-G-T^P-G-G).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: IKKα (phospho-Thr23) antibody detects endogenous levels of IKKα only when phosphorylated at threonine 23.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : O15111
References: Yuan ZQ, et al.(2002) J Biol Chem; 277(33): 29973-82.
Ozes ON, et al. (1999) Nature; 401(6748): 82-5.



P-Peptide - +

Immunohistochemical analysis of paraffin- embedded human colon carcinoma tissue, using IKKα (phospho-Thr23) antibody (E011129).

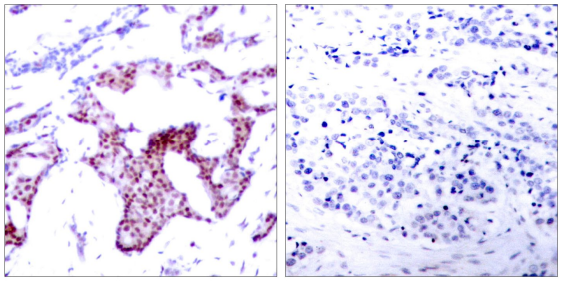


Western blot analysis of extracts using IKKα (phospho-Thr23) antibody (E011129).

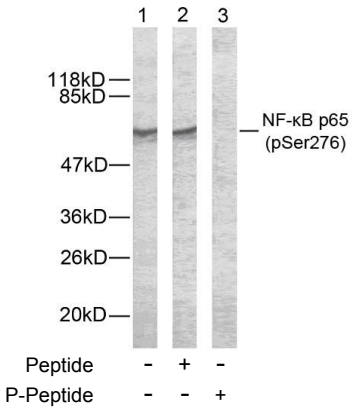
NF-κB p65 (Phospho-Ser276) Antibody

E011011

Catalog Number: E011011-1, E011011-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human NF-κB p65 around the phosphorylation site of serine 276 (R-P-S^P-D-R).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatogramphy using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: NF-κB p65 (phospho-Ser276) antibody detects endogenous levels of NF-κB p65 only when phosphorylated at serine 276.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100 IF:1:100~1:200
Swiss-Prot No. : Q04206
References: Baeuerle P A, et al. (1994) Annu Rev Immunol. 12:141-179.
Baeuerle P A, et al. (1996) Cell 87:13-20.
Haskill S, et al. (1991) Cell 65:1281-1289.



Immunohistochemical analysis of paraffin- embedded human breast carcinoma tissue using NF-κB p65 (phospho-Ser276) antibody (E011011).

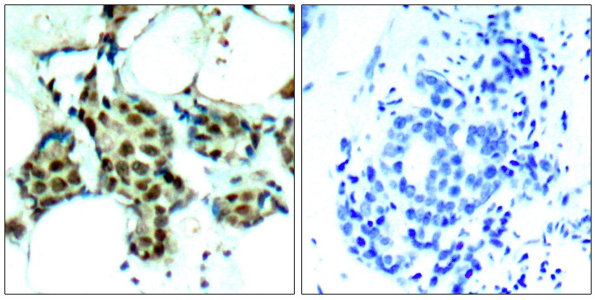


Western blot analysis of extract from HeLa cells using NF-κB p65 (phospho-Ser276) antibody (E011011).

p38 MAPK (Phospho-Tyr182) Antibody

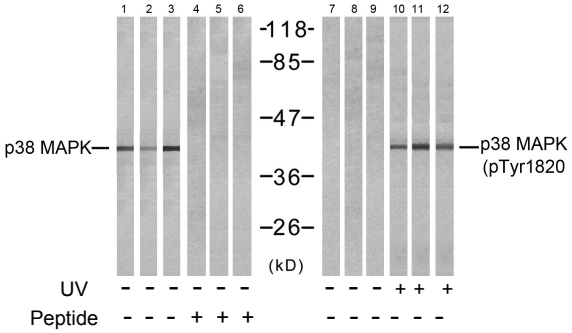
E011253

Catalog Number: E011253-1, E011253-2
Amount: 50μg/50μl, 100μg/100μl
Swiss-Prot No. : Q16539
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human p38 MAPK around the phosphorylation site of tyrosine 182 (T-G-Y^{P}-V-A).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: p38 MAPK (phospho-Tyr182) antibody detects endogenous levels of P38MAPK only when phosphorylated at tyrosine 182.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50-1:100
References: Ming Zheng, et al.(2005) The FASEB Journal. 19: 109-111
Bernt van den et al.(2001) Blink Immunology, 166: 582-587
Arshad Rahman, et al. (2004) Am J Physiol Lung Cell Mol Physiol 287: L1017-L1024
Osamu Yoshino, et al. (2003) Endocrinology & Metabolism Vol. 88: 2236-2241



P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue, using P38 MAPK (phospho- Tyr182) antibody (E011253).

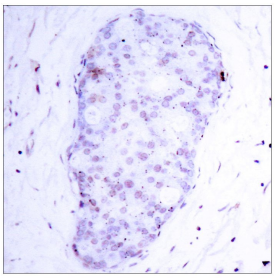


Western blot analysis of extracts from NIH-3T3 (Line 1, 4, 7 and 10) and cos7 (Line 2, 5, 8 and 11 and K562 (Line 3, 6, 9 and 12) cells, untreated or treated with UV (20min), using P38 MAPK (Ab-182) antibody (E021245, Lane 1, 2, 3, 4, 5 and 6) and P38 MAPK (phospho- Tyr182) antibody (E011253, Lane 7, 8, 9, 10, 11 and12).

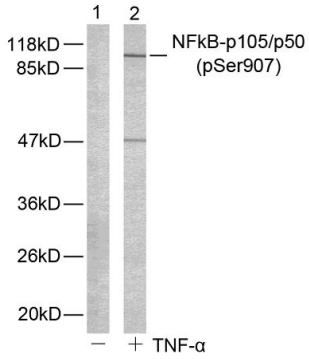
NF-κB p105/p50 (Phospho-Ser907) Antibody

E011019

Catalog Number: E011019-1, E011019-2
Amount: 50μg/50μl, 100μg/100μl
Form of Antibody: Rabbit IgG in phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Storage/Stability: Store at -20°C/1 year
Immunogen: The antiserum was produced against synthesized phosphopeptide derived from human NF-κB p105/p50 around the phosphorylation site of serine 907 (P-L-S^P-P-A).
Purification: The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Specificity/Sensitivity: NF-κB p105/p50 (phospho-Ser907) antibody detects endogenous levels of NF-κB p105/p50 only when phosphorylated at serine 907.
Reactivity: Human
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : P19838
References:
Hou S, et al. (2003) J Biol Chem. 278(46): 45994-45998.
Baeuerle P A, et al. (1994) Annu Rev Immunol. 12:141-179.
Baeuerle P A, et al. (1996) Cell 87:13-20.
Haskill S, et al. (1991) Cell 65:1281-1289.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using NF-κB p105/p50 (phospho-Ser907) antibody (E011019).



Western blot analysis of extract from HeLa cells untreated or treated with TNF-α using NF-κB p105/p50 (phospho-Ser907) antibody (E011019).