Phospho-MAPK Pathway Sampler Kit

E051018

Kits Includes	Cat.	Quantity	Application	Reactivity	Source
ATF2 (Phospho-Thr71 or 53) Antibody	E011031-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
P38 MAPK(Phospho-Tyr182) Antibody	E011253-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
p53 (Phospho-Ser15) Antibody	E011094-1	50μg/50μl	IHC, WB, IF	Human	Rabbit
STAT1 (Phospho-Tyr701) Antibody	E011044-1	50μg/50μl	IHC, WB	Human, Mouse, Rat	Rabbit
Tau (Phospho-Ser262) Antibody	E011111-1	50μg/50μl	WB	Human, Mouse, Rat	Rabbit

ATF2 gene encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds to the cAMP-responsive element (CRE), an octameric palindrome. The protein forms a homodimer or heterodimer with c-Jun and stimulates CRE-dependent transcription. The protein is also a histone acetyltransferase (HAT) that specifically acetylates histones H2B and H4 in vitro; thus it may represent a class of sequence-specific factors that activate transcription by direct effects on chromatin components. Additional transcript variants have been identified but their biological validity has not been determined. Transcriptional activator, probably constitutive, which binds to the cAMP-responsive element (CRE) (consensus: 5'-GTGACGT[AC][AG]-3'), a sequence present in many viral and cellular promoters. Interaction with JUN redirects JUN to bind to CRES preferentially over the 12-O-tetradecanoylphorbol-13-acetate response elements (TRES) as part of an **ATF2**-c-Jun complex.

MAPK14 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).

TP53 gene encodes tumor protein **p53**, which responds to diverse cellular stresses to regulate target genes that induce cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. **p53** protein is expressed at low level in normal cells and at a high level in a variety of transformed cell lines, where it's believed to contribute to transformation and malignancy. **p53** is a DNA-binding protein containing transcription activation, DNA-binding, and oligomerization domains. It is postulated to bind to a **p53**-binding site and activate expression of downstream genes that inhibit growth and/or invasion, and thus function as a tumor suppressor. Mutants of **p53** that frequently occur in a number of different human cancers fail to bind the consensus DNA binding site, and hence cause the loss of tumor suppressor activity. Alterations of this gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome. Multiple **p53** variants due to alternative promoters and multiple alternative splicing have been found. These variants encode distinct isoforms, which can regulate **p53** transcriptional activity. Acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. One of the activated genes is an inhibitor of cyclin-dependent kinases. Apoptosis induction seems to be mediated either by stimulation of BAX and FAS antigen expression, or by repression of Bcl-2 expression. Implicated in Notch signaling cross-over.

p53 (aka TP53) is a transcription factor whose protein levels and post-translational modification state alter in response to cellular stress (such as DNA damage, hypoxia, spindle damage). Activation of p53 begins through a number of mechanisms including phosphorylation by ATM, ATR, Chk1 and MAPKs. MDM2 is a ubiquitn ligase that binds p53 and targets p53 for proteasomal degradation. Phosphorylation, p14ARF and USP7

prevent MDM2-p53 interactions, leading to an increase in stable p53 tetramers in the cytoplasm. Further modifications such as methylation and acetylation lead to an increase in p53 binding to gene specific response elements. p53 regulates a large number of genes (>100 genes) that control a number of key tumor suppressing functions such as cell cycle arrest, DNA repair, senescence and apoptosis. Whilst the activation of p53 often leads to apoptosis, p53 inactivation facilitates tumor progression.

STAT1 is a member of the **STAT** protein family. In response to cytokines and growth factors, **STAT** family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein can be activated by various ligands including interferon-alpha, interferon-gamma, EGF, PDGF and IL6. This protein mediates the expression of a variety of genes, which is thought to be important for cell viability in response to different cell stimuli and pathogens. Two alternatively spliced transcript variants encoding distinct isoforms have been described. Signal transducer and activator of transcription that mediates signaling by interferons (IFNs). Following type I IFN (IFN-alpha and IFN-beta) binding to cell surface receptors, Jak kinases (TYK2 and JAK1) are activated, leading to tyrosine phosphorylation of **STAT**1 and **STAT**2. The phosphorylated **STAT**s dimerize, associate with ISGF3G/IRF-9 to form a complex termed ISGF3 transcription factor, that enters the nucleus. ISGF3 binds to the IFN stimulated response element (ISRE) to activate the transcription of interferon stimulated genes, which drive the cell in an antiviral state. In response to type II IFN (IFN-gamma), **STAT**1 is tyrosine- and serine-phosphorylated. It then forms a homodimer termed IFN-gamma-activated factor (GAF), migrates into the nucleus and binds to the IFN gamma activated sequence (GAS) to drive the expression of the target genes, inducing a cellular antiviral state.

STATs (signal transducers and activators of transcription) are a family of 7 transcription factors that form part of the JAK-STAT signaling cascade. This cascade is the basis of the signal transduction mechanism for many cytokine receptors. Once activated by phosphorylation by JAKs, STATs translocate to the nucleus. Accumulation of STATs in the nucleus is both rapid and tightly controlled. A number of factors regulate the JAK-STAT pathway including STAT dephosphorylation by phosphatases, altered nuclear import-export dynamics of STAT, and STAT gene activation antagonists such as SOCS (suppressors of cytokine signaling) and PIAS (Protein Inhibitors of Activated STATs).

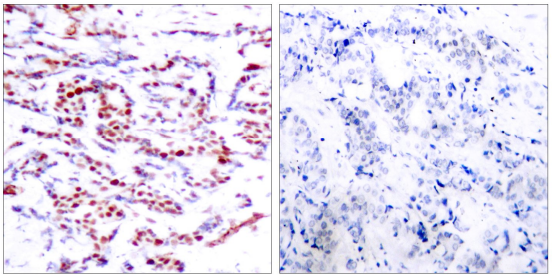
MAPT gene encodes the microtubule-associated protein **tau** (MAPT) whose transcript undergoes complex, regulated alternative splicing, giving rise to several mRNA species. MAPT transcripts are differentially expressed in the nervous system, depending on stage of neuronal maturation and neuron type. MAPT gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy. Promotes microtubule assembly and stability, and might be involved in the establishment and maintenance of neuronal polarity. The C-terminus binds axonal microtubules while

the N-terminus binds neural plasma membrane components, suggesting that **tau** functions as a linker protein between both. Axonal polarity is predetermined by **tau** localization (in the neuronal cell) in the domain of the cell body defined by the centrosome. The short isoforms allow plasticity of the cytoskeleton whereas the longer isoforms may preferentially play a role in its stabilization.

ATF-2 (Phospho-Thr71 or 53) Antibody

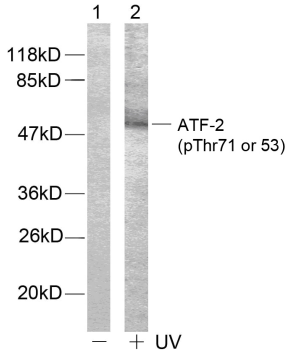
E011031

Catalog Number: E011031-1, E011031-2
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 ATF-2 around the phosphorylation site of threonine 71 or 53 (T-P-T^{P}-P-T).
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: ATF-2 (phospho-Thr71 or 53) antibody detects endogenous levels of ATF-2 only when phosphorylated at threonine 71 or 53.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : P15336
References:
Sevilla A, et al. (2004) J Biol Chem. 279(26):27458-27465.
Waetzig G H, et al. (2002) J Immunol. 168(10): 5342-5351.
Abdel-Hafiz H A, et al. (1992) Mol Endocrinol. 6: 2079-2089.
Gupta S, et al. (1995) Science. 267: 389-393.
Van Dam H, et al. (1995) EMBO J. 14(8): 1798-1811.



P-Peptide - +

Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using ATF-2 (phospho-Thr71 or 53) antibody (E011031).

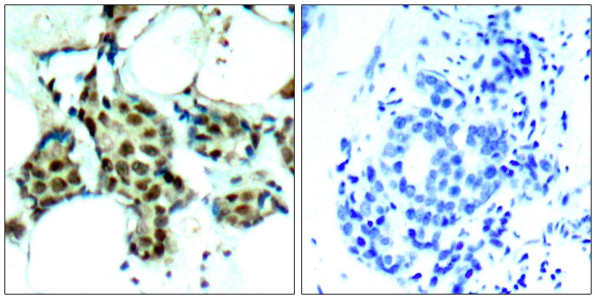


Western blot analysis of extract from HeLa cells, using ATF-2 (phospho-Thr71 or 53) antibody (E011031).

p38 MAPK (Phospho-Tyr182) Antibody

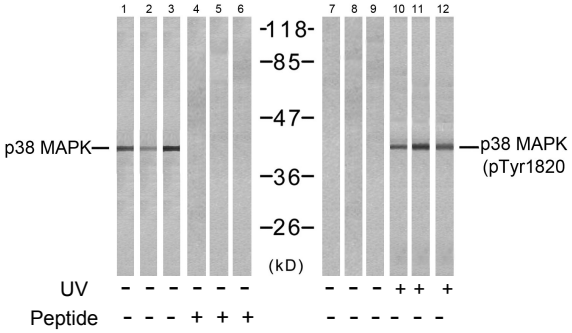
E011253

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).

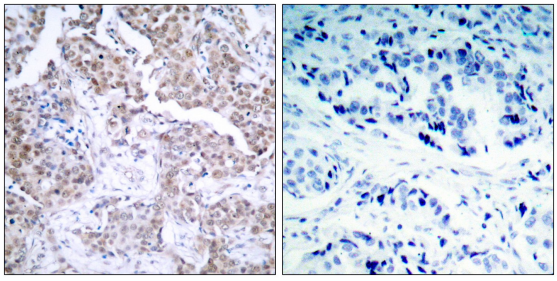


Western blot analysis of extracts from NIH-3T3 (Line 1, 4, 7 and 10) and cos7 (Line 2, 5, 8 and 11 and K562 (Line 3, 6, 9 and 12) cells, untreated or treated with UV (20min), using P38 MAPK (Ab-182) antibody (E021245, Lane 1, 2, 3, 4, 5 and 6) and P38 MAPK (phospho- Tyr182) antibody (E011253, Lane 7,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

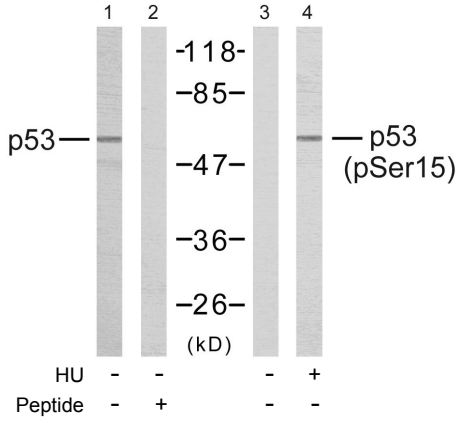
p53 (Phospho-Ser15) Antibody

E011094

Catalog Number: E011094-1, E011094-2
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 p53 around the phosphorylation site of serine 15 (P-L-S^P-Q-E).
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: p53 (phospho-Ser15) antibody detects endogenous levels of p53 only when phosphorylated at serine15.
Reactivity: Human
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100 IF: 1:100-200
Swiss-Prot No. : P04637
References:
Shieh, S. Y. et al. (1999) *EMBO J.* 18, 1815-1823.
Honda, R. et al. (1997) *FEBS Lett.* 420, 25-27.
Hirao, A. et al. (2000) *Science* 287, 1824-1827.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using p53 (phospho-Ser15) antibody (E011094).

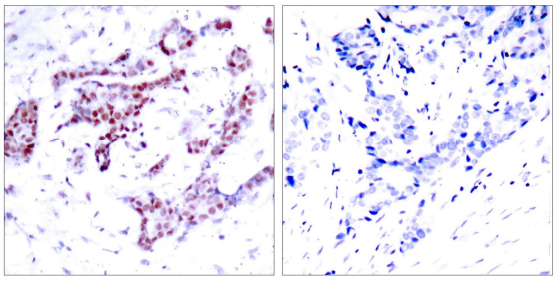


Western blot analysis of the extracts from HeLa cells untreated or treated with hydroxyurea, using p53 (Ab-15) antibody (E021085, Line1 and 2) and p53 (phospho-Ser15) antibody (E011094, Line3 and 4).

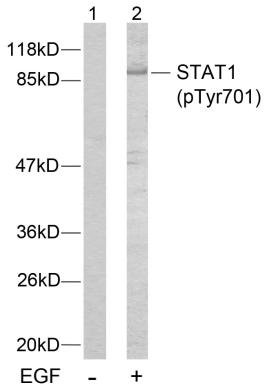
STAT1 (Phospho-Tyr701) Antibody

E011044

Catalog Number: E011044-1, E011044-2
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 STAT1 around the phosphorylation site of tyrosine 701 (T-G-Y^{P}-I-K).
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: STAT1 (phospho-Tyr701) antibody detects endogenous levels of STAT1 only when phosphorylated at tyrosine 701.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000 IHC: 1:50~1:100
Swiss-Prot No. : P42224
References:
Heim M H, (1999) J Recept Signal Transduct Res. 19: 75-120.
Durbin J E, et al. (1996) Cell. 84: 443-450.
Demoulin J, B. et al. (1999) J Biol Chem. 274: 25855-258.
Ihle J N, et al. (1994) Trends Biochem Sci. 19: 222-227.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using STAT1 (phospho-Tyr701) antibody (E011044).

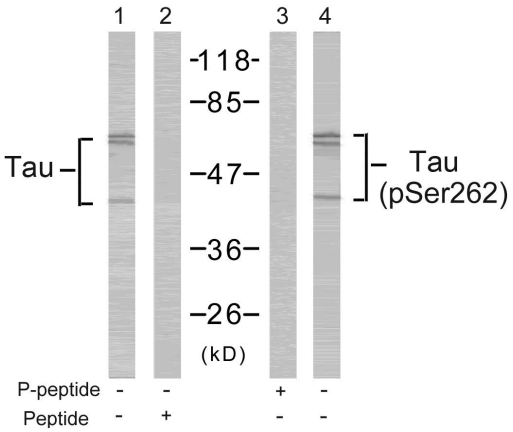


Western blot analysis of extracts from MCF7 cells using STAT1 (phospho-Tyr701) antibody (E011044).

Tau (Phospho-Ser262) Antibody

E011111

Catalog Number: E011111-1, E011111-2
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 Tau around the phosphorylation site of serine 262 (I-G-S^P-T-E).
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: Tau (phospho-Ser262) antibody detects endogenous levels of Tau only when phosphorylated at serine 262.
Reactivity: Human, Mouse, Rat
Applications: WB: 1:500~1:1000
Swiss-Prot No. : P10636
References: Timm T, et al. (2003) EMBO J; 22(19): 5090-5101.



Western blot analysis of extracts from mouse brain tissue using Tau (Ab-262) antibody (E021100, Lane 1 and 2) and Tau (phospho-Ser262) antibody (E011111, Lane 3 and 4).