abcam

Product datasheet

STAT3 Transcription Factor Assay Kit (Colorimetric) ab207229

5 References 1 Image

Overview

Product name	STAT3 Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	< 600 ng/well
Assay time	3h 30m
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	STAT3 Transcription Factor Assay Kit (Colorimetric) (ab207229) is a high throughput assay to quantify STAT3 activation in nuclear extracts. 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 STAT3 consensus binding site (5' – TTCCCGGAA – 3') has been immobilized onto a 96-well plate. Active STAT3 present in the nuclear extract specifically binds to the oligonucleotide. STAT3 is detected by a primary antibody that recognizes an epitope of STAT3 accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout that at OD 450 nm. This product detects human, mouse and rat STAT3. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 0.6 µg nuclear extract/well. Detection range: 0.3 – 10 µg nuclear extract/well.
Notes	STAT (signal transducers and activators of transcription) transcription factors were discovered fourteen years ago as mediators of interferon-induced gene expression. They comprise a family of latent cytoplasmic proteins that are activated to participate in gene control when cells encounter various extracellular polypeptides. Their critical role in development and normal cell signaling has

been largely determined through the analysis of transgenic mice lacking individual STAT genes. The STAT family consists of seven members that are activated by virtually every cytokine and growth factor.

The STAT proteins are unique among transcription factors in containing an SH2 (src-homology 2), phosphotyrosine-binding domain, a common protein-protein interaction domain among signaling proteins. Tyrosine phosphorylation around residue 700 is essential for the dimerization of STATs and the concomitant nuclear translocation of the dimer. Ligand-activated receptors that catalyze this phosphorylation include receptors with intrinsic tyrosine kinase activity (epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and colony-stimulating factor-1) as well as receptors that lack intrinsic tyrosine kinase activity but to which Janus kinases (JAKs) are noncovalently associated. Receptors to which JAKs are bound are often referred to as cytokine receptors. Their ligands include IFN-α, -β and -γ; interleukins (IL) 2 to 7, 10 to 13, and 15; and erythropoietin, growth hormone, prolactin, thrombopoietin and other polypeptides. STAT dimers and heterodimers, but not monomers, are competent to bind DNA. The known DNA binding heterodimers are STAT1:2 (strong binding requires the joint presence of another protein, p48) and STAT1:3. STATs that form homodimers that bind DNA include STAT 1, 3, 4, 5 (STAT5A and 5B interact in a manner equivalent to a heterodimer) and 6.

In most cases, STAT activation is transient. Inactivation of STAT proteins is carried out by several mechanisms, including dephosphorylation of STAT proteins in the nucleus and degradation through the ubiquitin-proteosome pathway. A novel family of negative feedback inhibitors of the JAK-STAT pathway has been identified, referred to as suppressor-of-cytokine-signaling (SOCS) proteins/JAK binding (JAB) proteins, and STAT-induced STAT inhibitors (SSIs). In addition, a family of protein inhibitors of activated STAT (PIAS) proteins has been identified.

Platform Microplate reader

Properties

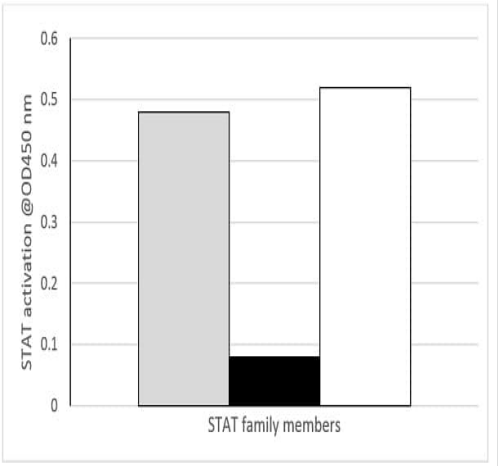
Storage instructions Please refer to protocols.

Components	1 x 96 tests	5 x 96 tests
10X Antibody Binding Buffer	1 x 2.2ml	1 x 11ml
10X Wash Buffer	1 x 22ml	1 x 110ml
96-well IRF-3 assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	1 x 55µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
Hep G2 (IL-6, 100 ng/ml) nuclear extract	1 x 40µl	1 x 200µl
Herring sperm DNA	1 x 100µl	1 x 500µl
Lysis Buffer	1 x 10ml	1 x 50ml

Components	1 x 96 tests	5 x 96 tests
Plate sealer	1 unit	5 units
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
STAT mutated oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl
STAT Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl
STAT3 antibody	1 x 20µl	1 x 100µl
Stop Solution	1 x 11ml	1 x 55ml

Function	Signal transducer and transcription activator that mediates cellular responses to interleukins, KITLG/SCF, LEP and other growth factors. Once activated, recruits coactivators, such as NCOA1 or MED1, to the promoter region of the target gene (PubMed:17344214). May mediate cellular responses to activated FGFR1, FGFR2, FGFR3 and FGFR4. Binds to the interleukin-6 (IL-6)-responsive elements identified in the promoters of various acute-phase protein genes. Activated by IL31 through IL31RA. Involved in cell cycle regulation by inducing the expression of key genes for the progression from G1 to S phase, such as CCND1 (PubMed:17344214). Mediates the effects of LEP on melanocortin production, body energy homeostasis and lactation (By similarity). May play an apoptotic role by transctivating BIRC5 expression under LEP activation (PubMed:18242580). Cytoplasmic STAT3 represses macroautophagy by inhibiting EIF2AK2/PKR activity.
Tissue specificity	Heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas.
Involvement in disease	Hyperimmunoglobulin E recurrent infection syndrome, autosomal dominant Autoimmune disease, multisystem, infantile-onset
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Tyrosine phosphorylated upon stimulation with EGF. Tyrosine phosphorylated in response to constitutively activated FGFR1, FGFR2, FGFR3 and FGFR4 (By similarity). Activated through tyrosine phosphorylation by BMX. Tyrosine phosphorylated in response to IL6, IL11, LIF, CNTF, KITLG/SCF, CSF1, EGF, PDGF, IFN-alpha, LEP and OSM. Activated KIT promotes phosphorylation on tyrosine residues and subsequent translocation to the nucleus. Phosphorylated on serine upon DNA damage, probably by ATM or ATR. Serine phosphorylation is important for the formation of stable DNA-binding STAT3 homodimers and maximal transcriptional activity. ARL2BP may participate in keeping the phosphorylated state of STAT3 within the nucleus. Upon LPS challenge, phosphorylated within the nucleus by IRAK1. Upon erythropoietin treatment, phosphorylated on Ser-727 by RPS6KA5. Phosphorylation at Tyr-705 by PTK6 or FER leads to an increase of its transcriptional activity. Dephosphorylation on tyrosine residues by PTPN2 negatively regulates IL6/interleukin-6 signaling.
Cellular localization	Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Translocated into the nucleus upon tyrosine phosphorylation and dimerization, in response to signaling by activated FGFR1, FGFR2, FGFR3 or FGFR4. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. Identified in a complex with LYN and PAG1.

Images



TYPICAL DATA

Nuclear extracts from HepG2 cells treated with IL-6 (100 ng/ml) were assayed for STAT3 activation in the absence (grey) or presence of wild-type (black) or mutated (white) consensus binding oligonucleotides. These results are provided for demonstration purposes only.

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