abcam

Product datasheet

STAT1 (pS727) ELISA Kit ab126454

5 Images

Overview

Product name	STAT1 (pS727) ELISA Kit
Detection method	Colorimetric
Sample type	Cell Lysate
Assay type	Semi-quantitative
Assay time	5h 00m
Assay duration	Multiple steps standard assay
Species reactivity	**Reacts with:** Mouse, Human
Product overview	ab126454 is a very rapid, convenient and sensitive assay kit that can monitor the activation or function of important biological pathways in cell lysates. By determining phosphorylated STAT1 protein in your experimental model system, you can verify pathway activation in your cell lysates. You can simultaneously measure numerous different cell lysates without spending excess time and effort in performing a Western Blot analysis. This Sandwich ELISA kit is an in vitro enzyme-linked immunosorbent assay for the measurement of human and mouse phospho-STAT1 (Ser727). An anti-STAT1 (Ser727) antibody has been coated onto a 96-well plate. Samples are pipetted into the wells and phosphorylated STAT1 (Ser727) present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-STAT1 antibody is used to detect phosphorylated STAT1. After washing away unbound antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of STAT1 (Ser727) bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.
Notes	Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.
Platform	Microplate

Properties

Storage instructions	Store at -20°C. Please refer to protocols.

Components	1 x 96 tests
20X Wash Buffer	1 x 25ml
2X Cell Lysis Buffer	1 x 5ml
5X Assay Diluent	1 x 15ml
Detection Antibody anti-pan STAT1	2 vials
HRP-Streptavidin Concentrate 40x concentrated HRP-conjugated streptavidin	2 x 200µl
Positive Control: lyophilized powder from A431 cell lysate	1 vial
STAT1 (Ser727) Microplate (12 strips x 8 wells) coated with anti-STAT1 (Ser727)	1 unit
Stop Solution	1 x 8ml
TMB One-Step Substrate Reagent	1 x 12ml

Function

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 STAT1 and STAT2. The phosphorylated STATs 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), STAT1 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.

Involvement in disease

Note=STAT1 deficiency results in impaired immune response leading to severe mycobacterial and viral diseases. In the case of complete deficiency, patients can die of viral disease. Defects in STAT1 are a cause of mendelian susceptibility to mycobacterial disease (MSMD) [MIM:209950]; also known as familial disseminated atypical mycobacterial infection. This rare condition confers predisposition to illness caused by moderately virulent mycobacterial species, such as Bacillus Calmette-Guerin (BCG) vaccine and environmental non-tuberculous mycobacteria, and by the more virulent Mycobacterium tuberculosis. Other microorganisms rarely cause severe clinical disease in individuals with susceptibility to mycobacterial infections, with the exception of Salmonella which infects less than 50% of these individuals. The pathogenic mechanism underlying MSMD is the impairment of interferon-gamma mediated immunity whose severity determines the clinical outcome. Some patients die of overwhelming mycobacterial disease with lepromatous-like lesions in early childhood, whereas others develop, later in life, disseminated but curable infections with tuberculoid granulomas. MSMD is a genetically heterogeneous disease with autosomal recessive, autosomal dominant or X-linked inheritance.

Sequence similarities

Belongs to the transcription factor STAT family.
Contains 1 SH2 domain.

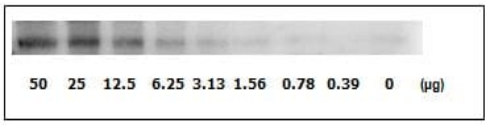
Post-translational modifications

Phosphorylated on tyrosine and serine residues in response to IFN-alpha, IFN-gamma, PDGF and EGF. Phosphorylation on Tyr-701 (lacking in beta form) by JAK promotes dimerization and subsequent translocation to the nucleus. Phosphorylation on Ser-727 by several kinases including MAPK14, ERK1/2 and CAMKII on IFN-gamma stimulation, regulates STAT1 transcriptional activity. Phosphorylation on Ser-727 promotes sumoylation though increasing interaction with

PIAS. Phosphorylation on Ser-727 by PKCdelta induces apoptosis in response to DNA-damaging agents.
Sumoylated by SUMO1, SUMO2 and SUMO3. Sumoylation is enhanced by IFN-gamma-induced phosphorylation on Ser-727, and by interaction with PIAS proteins. Enhances the transactivation activity.
ISGylated.

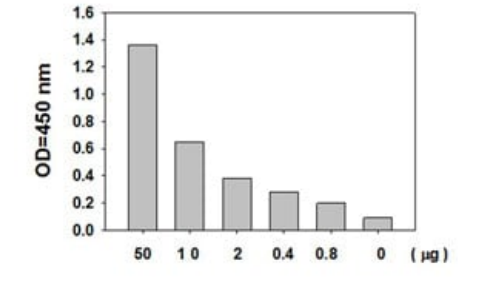
Cellular localization Cytoplasm. Nucleus. Translocated into the nucleus in response to IFN-gamma-induced tyrosine phosphorylation and dimerization.

Images



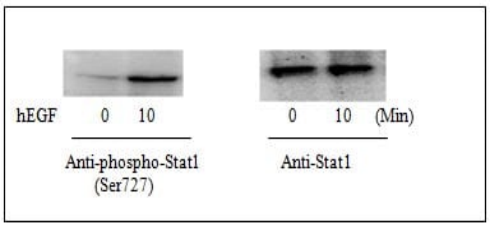
Western blot - STAT1 (pS727) ELISA Kit (ab126454)

The A431 cells were treated with100 ng/ml recombinant human EGF for 20 minutes. Serial dilutions of lysates were analyzed by Western blot. Immunoblots were incubated with anti-phospho-STAT1 (Ser727).



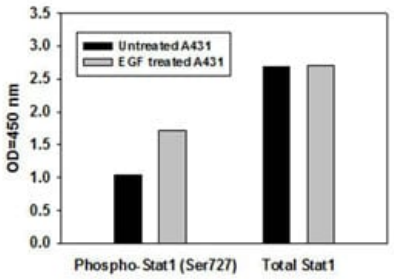
Sandwich ELISA - STAT1 (pS727) ELISA Kit (ab126454)

The A431 cells were treated with100 ng/ml recombinant human EGF for 20 minutes. Serial dilutions of lysates were analyzed in this ELISA.



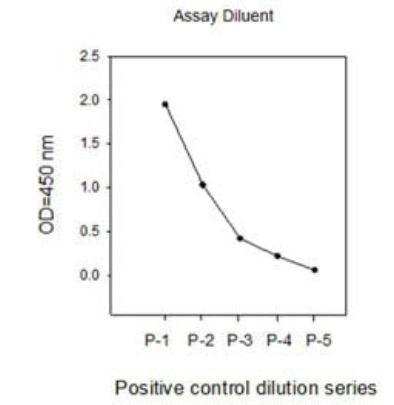
Western blot - STAT1 (pS727) ELISA Kit (ab126454)

A431 cells were treated or untreated with 100 ng/ml recombinant human EGF for 10 min. Cell lysates were analyzed by Western Blot.



Sandwich ELISA - STAT1 (pS727) ELISA Kit (ab126454)

A431 cells were treated or untreated with 100 ng/ml recombinant human EGF for 10 min. Cell lysates were analyzed using this phosphoELISA.



Sandwich ELISA - STAT1 (pS727) ELISA Kit (ab126454)

A431 cells were treated with recombinant human EGF at 37°C for 20 min. Solubilize cells at 4 x 10^7 cells/ml in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA.

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