abcam

Product datasheet

STAT1 (pY701) + total GAPDH PhosphoTracer ELISA Kit ab119671

SimpleStep ELISA®

5 图像

概述

产品名称	STAT1 (pY701) + total GAPDH PhosphoTracer ELISA试剂盒
检测方法	Fluorescent
样品类型	Cell culture extracts
检测类型	Semi-quantitative
检测时间	2h 0m
实验步骤	One step assay
种属反应性	**与反应:** Human
产品概述	PhosphoTracer assays use a traditional immuno-sandwich format, but with a major difference **both the analyte and the assay reagents are added to the PhosphoTracer assay microplate at the same time**. After a short incubation period, unbound assay reagents and analytes are washed away, and immuno-complexes containing both antibodies are detected. The process can take as little as 60 minutes to complete. PhosphoTracer kits also allow a higher degree of assay flexibility. In contrast to other ELISA formats, no antibodies are present on the assay microplate itself, so assays for several different targets can be performed in different wells on the same microplate. Simply mix the lysate with your target reagents of choice, using the microplate configuration of your choice. A whole new way of performing cellular assays, PhosphoTracer takes the hard work out of running a standard ELISA, while still giving the high quality results expected from a sandwich immunoassay. Fully self-contained kits are supplied in convenient 96-well packs. Simple to use and highly sensitive PhosphoTracer kits are designed to get results, fast. Abcam's PhosphoTracer **STAT1** (p-Tyr701) assays detect endogenous levels of phosphorylated **STAT1** (GenBank Accession NP_009330) in cellular lysates. The phospho-STAT1 assay detects **STAT1** only when phosphorylated at Tyr701. The **GAPDH** assay detects endogenous levels of **GAPDH** (GenBank Accession NP_002037) in cellular lysates. The substrate used with the HRP conjugated detection antibody is a combination of 10-Acetyl-3,7-dihydroxyphenoxazine (ADHP) (wavelength exc/em = 530-540nm / 590-600nm), a highly

sensitive and stable substrate for HRP) and ADHP Dilution Buffer (a stabilized H_2O_2 solution).

Learn more about the fluorogenic substrate, ADHP.

说明

Sensitivity: Phospho-STAT1: 2,000 cells/well (tested in HeLa cells), GAPDH: 25 pg/ml (tested with rhGAPDH).

Range: Phospho-STAT1: 2,000-100,000 cells/well tested (tested in HeLa cells), GAPDH: 25-6,000 pg/ml (tested with rhGAPDH).

经测试应用

适用于: Sandwich ELISA

平台

Microplate

性能

存放说明

Store at +4°C. Please refer to protocols.

Components	
Lysis Buffer (5X)	1 x 15ml
Wash Buffer (10X)	1 x 15ml
ADHP Dilution Buffer	1 x 15ml
ADHP (100X)	1 x 120µl
Enhancer Solution	1 x 1ml
Stop Solution	1 x 2ml
Assay Control Lysate (lyophilized)	1 x 0.25ml
96-well PhosphoTracer assay plate (stripwell)	1 x unit
Adherent plate seal	2 x unit
Rabbit monoclonal GAPDH (24 assay points)	1 x 0.75ml
Mouse monoclonal GAPDH (HRP) (24 assay points)	1 x 0.75ml
Rabbit monoclonal Phospho-STAT1 (Tyr701) (72 assay points)	3 x 0.75ml
Rabbit monoclonal Phospho-STAT1 (HRP) (72 assay points)	3 x 0.75ml

功能

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.

疾病相关	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.
序列相似性	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
翻译后修饰	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.
细胞定位	Cytoplasm. Nucleus. Translocated into the nucleus in response to IFN-gamma-induced tyrosine phosphorylation and dimerization.

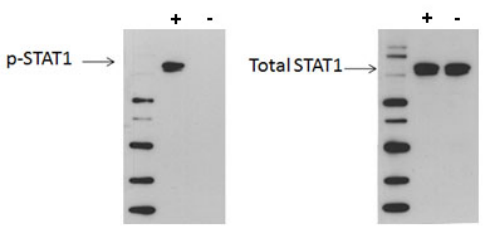
应用

Our Abpromise guarantee covers the use of **ab119671** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

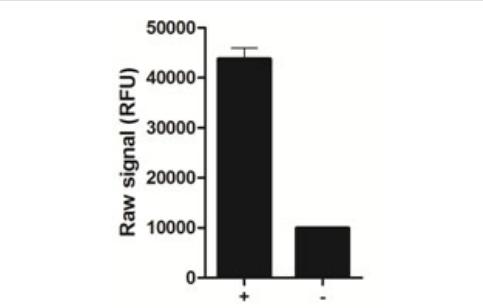
应用	Ab评论	说明
Sandwich ELISA		Use at an assay dependent concentration. Phospho-STAT1: Tested in Hela; GAPDH: Tested in HeLa, A431, A549, PC3, U2OS.

图片



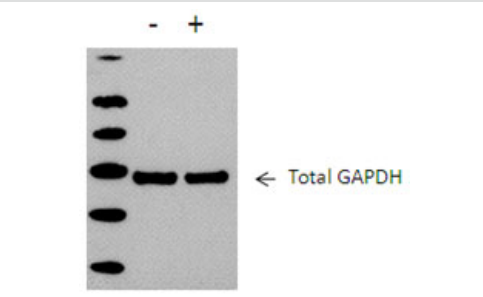
Western blot - PhosphoTracer STAT1 (pT701) + total GAPDH ELISA Kit (ab119671)

Using Western blot, STAT1 phosphorylation at Tyr701 is detected in interferon-gamma-treated HeLa cells (+), compared with untreated HeLa cells (-).



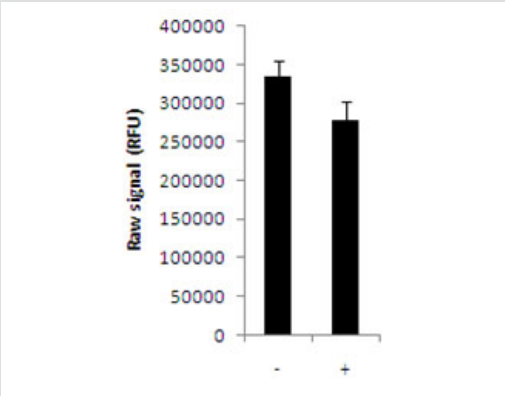
Sandwich ELISA - PhosphoTracer STAT1 (pT701) + total GAPDH ELISA Kit (ab119671)

Using the STAT1 assay kit, STAT1 phosphorylation at Tyr701 is detected in interferon-gamma-treated HeLa cells (+), compared with untreated HeLa cells (-).



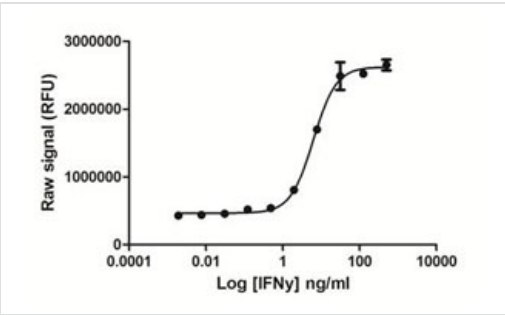
Western blot - PhosphoTracer STAT1 (pT701) + total GAPDH ELISA Kit (ab119671)

Using Western blot, cellular GAPDH is readily detected in A431 cellular lysates, in either untreated cells (-), or cells treated with EGF (+).



Sandwich ELISA - PhosphoTracer STAT1 (pT701) + total GAPDH ELISA Kit (ab119671)

Using the GAPDH assay kit, cellular GAPDH is readily detected in A431 cellular lysates, in either untreated cells (-), or cells treated with EGF (+).



Sandwich ELISA - PhosphoTracer STAT1 (pT701) + total GAPDH ELISA Kit (ab119671)

HeLa cells were seeded at 40K cells/well in a 96 well tissue culture microplate overnight. The next day cells were treated with various concentrations of interferon-gamma for 20 mins. The medium was removed from the wells, and cells were lysed with 120 µl/well of Lysis Mix, with shaking for 10 min. The lysates were transferred to a PhosphoTracer assay plate and assayed for phospho-STAT1, using the standard protocol. Signal in the wells was determined using a plate reader.

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