abcam

Product datasheet

Phospho-STAT2 (Y689) + Total In-Cell ELISA Kit ab207486

Overview

Product name	Phospho-STAT2 (Y689) + Total In-Cell ELISA Kit
Detection method	Colorimetric
Sample type	Adherent cells, Suspension cells
Assay type	Cell-based (quantitative)
Assay duration	Multiple steps standard assay
Species reactivity	**Reacts with:** Mouse
Product overview	Phospho-STAT2 (Y689) + Total In-Cell ELISA Kit (ab207486) provides a simple, efficient, cell-based method to monitor proteins activated by phosphorylation. The kit is designed specifically to quantify activated (phosphorylated) STAT2 and/or total STAT2. Cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are rapidly fixed to preserve activation-specific protein modifications. Each well is then incubated with a primary antibody that recognizes either phosphorylated STAT2 or total STAT2. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric readout. The relative number of cells in each well is then determined using the provided Crystal Violet solution. The 96-well plate format is suitable for high-throughput screening applications. The Phospho-STAT2 (Y689) + Total In-Cell ELISA Kit contains two 96-well plates and two primary antibodies. The phospho-STAT2 antibody is specific for phosphorylated STAT2 and was raised against a synthetic phospho-peptide corresponding to residues surrounding Tyrosine 689 of mouse STAT2. The total-STAT2 antibody recognizes STAT2 proteins regardless of the phosphorylation state. The kit can be used to study phosphorylated STAT2 relative to cell number or to determine STAT2 phosphorylation relative to the total STAT2 protein found in the cells. Once the phospho-STAT2 and total-STAT2 signals have been normalized for cell number, a comparison of the ratio of phosphorylated STAT2 to total STAT2 for each of the cell growth conditions can be made. The provided total-STAT2 antibody can be used as a positive control to demonstrate that the cells contain STAT2, the kit reagents are functional and that the protocol is performed correctly. Also, because fixed cells are stable for several weeks, you can prepare many plates simultaneously and then perform the assay when desired.
Notes	STAT (signal transducers and activators of transcription) 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 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.

STAT proteins are involved in a wide variety of biological pathways. STAT1 is involved in the activation of IFNα and IFNγ genes, STAT2 in the activation of IFNα genes, STAT4 and STAT6 in T-helper cell development and STAT5 in milk production. Disruption of STAT functions in mouse leads to several defects such as immune deficiency (STAT1), embryonic lethality (STAT2), lack of gastrulation (STAT3), T-helper 1 cell dysfunction (STAT4), lack of lactation (STAT5A, 5B) and T-helper 2 cell dysfunction (STAT6). The disruption of STAT signaling blocks neoplastic transformation, thus making inhibitors of STAT proteins candidates for the treatment of cancer.

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).

Tested applications **Suitable for:** In-Cell ELISA

Platform Microplate

Properties

Storage instructions Please refer to protocols.

Components	1 x 96 tests	5 x 96 tests
1% SDS Solution	1 x 22ml	5 x 22ml
10% Triton X-100	1 x 10ml	5 x 10ml
10X PBS	1 x 120ml	5 x 120ml
1X Antibody Blocking Buffer	1 x 22ml	5 x 22ml
1X Antibody Dilution Buffer	1 x 30ml	5 x 30ml
96-well tissue culture plate	2 units	10 units
Anti-rabbit HRP-conjugated IgG	2 x 11µl	10 x 11µl
Crystal Violet Solution	1 x 22ml	5 x 22ml
Developing Solution	2 x 11ml	10 x 11ml

Components	1 x 96 tests	5 x 96 tests
Phospho-STAT2 antibody	1 x 9µl	5 x 9µl
Plate sealer	2 units	10 units
Stop Solution	2 x 11ml	10 x 11ml
Total-STAT2 antibody	1 x 9µl	5 x 9µl

Function	Signal transducer and activator of transcription that mediates signaling by type I IFNs (IFN-alpha and IFN-beta). Following type I IFN 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.
Sequence similarities	Belongs to the transcription factor STAT family. Contains 1 SH2 domain.
Post-translational modifications	Tyrosine phosphorylated in response to IFN-alpha.
Cellular localization	Cytoplasm. Nucleus. Translocated into the nucleus upon activation by IFN-alpha/beta.

Applications

Our Abpromise guarantee covers the use of **ab207486** in the following tested applications.

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

Application	Abreviews	Notes
In-Cell ELISA		Use at an assay dependent concentration.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors