abcam

Product datasheet

STAT (STAT1alpha, STAT3, STAT5A, STAT5B) Transcription Factor Assay Kit (Colorimetric) ab207228

2 References　画像数 1

医薬用外劇物

製品の概要

製品名	STAT (STAT1alpha, STAT3, STAT5A, STAT5B) Transcription Factor Assay Kit (Colorimetric)
検出方法	Colorimetric
サンプルの種類	Nuclear Extracts
アッセイタイプ	Semi-quantitative
検出感度	< 600 ng/well
全工程の試験時間	3h 30m
種交差性	**交差種:** Mouse, Rat, Human
製品の概要	The STAT1α, STAT5A and STAT5B antibodies can be used with human and rat extracts, while the STAT3 antibodies can be used with human, mouse and rat extracts. STAT (STAT1alpha, STAT3, STAT5A, STAT5B) Transcription Factor Assay Kit (Colorimetric) (ab207228) is a high throughput assay to quantify activation of STAT factors (STAT1alpha, STAT3, STAT5A, STAT5B) at the same time in one plate. 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 STAT (STAT1alpha, STAT3, STAT5A, STAT5B) consensus binding site (5' – TTCCCGGAA – 3') has been immobilized onto a 96-well plate. Active STAT (STAT1alpha, STAT3, STAT5A, STAT5B) present in the nuclear extract specifically binds to the oligonucleotide. STAT (STAT1alpha, STAT3, STAT5A, STAT5B) is detected by a primary antibody that recognizes an epitope of STAT (STAT1alpha, STAT3, STAT5A, STAT5B) 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 and rat STAT (STAT1alpha, STAT3, STAT5A, STAT5B). STAT3 can also be detected in mouse. 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.

特記事項

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 non-covalently 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 de-phosphorylation 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.

試験プラットフォーム

Microplate reader

製品の特性

保存方法

Please refer to protocols.

内容	2 x 96 tests
10X Antibody Binding Buffer	2 x 2.2ml
10X Wash Buffer	1 x 60ml
96-well IRF-3 assay plate	2 units
Anti-rabbit HRP-conjugated IgG	2 x 11µl
Binding Buffer	1 x 10ml
Developing Solution	2 x 11ml

内容	2 x 96 tests
Dithiothreitol (DTT) (1 M)	1 x 100µl
Herring sperm DNA	1 x 100µl
Lysis Buffer	1 x 10ml
Nb nuclear extract (prolactin stimulated)	1 x 40µl
Plate sealer	2 units
Protease Inhibitor Cocktail	1 x 100µl
STAT mutated oligonucleotide (10 pmol/µL)	1 x 100µl
STAT Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl
STAT1α antibody	1 x 11µl
STAT3 antibody	1 x 20µl
STAT5A antibody	1 x 11µl
STAT5B antibody	1 x 11µl
Stop Solution	1 x 60ml

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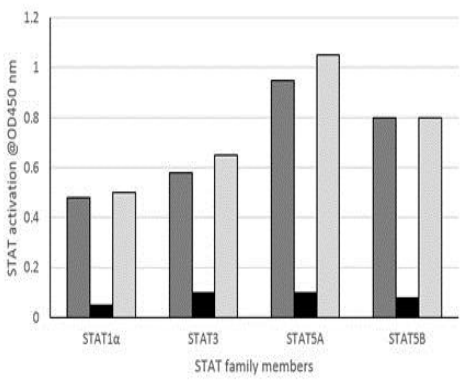
STAT3: Cytoplasm. Nucleus. Shuttles between the nucleus and the cytoplasm. Constitutive nuclear presence is independent of tyrosine phosphorylation. Predominantly present in the cytoplasm without stimuli. Upon leukemia inhibitory factor (LIF) stimulation, accumulates in the nucleus. The complex composed of BART and ARL2 plays an important role in the nuclear translocation and retention of STAT3. STAT 5A + B: Cytoplasm. Nucleus. Translocated into the nucleus in response to phosphorylation.

画像



Different amounts of Nb2 were tested for STAT Family activation.

Nuclear extracts from Nb2 cells stimulated with prolactin) were assayed for activity of STAT family members: STAT1alpha, STAT3, STAT5A, STAT5B in the absence (grey) or presence of wild-type (black) or mutated (white) consensus binding oligonucleotides. Note that the wild-type oligonucleotide reduces STAT binding by over 90% while incubation with the mutant STAT competitor oligo has limited effect on STAT binding to DNA. These results are provided for demonstration purposes only.

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