abcam

Product datasheet

c-Fos Transcription Factor Assay Kit (Colorimetric) ab207194

2 Images

Overview

Product name	c-Fos Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	< 1250 ng/well
Assay time	3h 30m
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	c-Fos Transcription Factor Assay Kit (Colorimetric) (ab207194) is a high throughput assay to quantify AP-1 c-Fos 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 TPA-responsive element (TRE) (5´–TGAGTCA– 3´) has been immobilized onto a 96-well plate. Activator protein-1 (AP1) present in the nuclear extract specifically binds to the oligonucleotide. AP1 family member c-Fos is detected by a primary antibody that recognizes an epitope of c-Fos accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout at OD 450 nm. This product detects human, mouse and rat c-Fos. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 1.25 µg nuclear extract/well. Detection range: 0.1 – 20 µg nuclear extract/well.
Notes	The activator protein-1 (AP1) transcription factors belong to a large family of structurally related transcription factors that includes ATF1-4, c-Fos, c-Jun, c-Myc and C/EBP. AP1 is composed of a mixture of heterodimeric complexes of proteins derived from the Fos and Jun families including c- Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD. Only Jun proteins can form transcriptionally

active homodimers within AP1 members, or heterodimers with CREB/ATF members, to bind the CRE element (5´ - TGACGTCA - 3´). Primarily, AP1 dimers bind to DNA on a TPA-response element (TRE) with the 5´ - TGA(C/G)TCA - 3´ sequence. Jun-Fos heterodimers form more stable complexes with TREs. These complexes display stronger transactivating activity than Jun-Jun homodimers.

Phosphorylation of AP1 family members by kinases is required for transactivation activity. For the Fos proteins, both N- and C-terminal domains flanking the bZIP domain require phosphorylation for biological activity.

AP1 expression is induced by multiple stimuli such as serum, growth factors, phorbol esters and oncogenes. These include peptide growth factors, cytokines of the TGF beta, TNF, and interferon families, neuronal depolarization and cellular stress. Upon serum starvation of human fibroblast cells, Fos and Jun protein production can be induced for up to 4 hours by adding serum. Interestingly, serum starvation lowers basal expression of FosB and c-Fos but has no significant effect on c-Jun.

AP1 proteins play a role in the expression of many genes involved in proliferation and cell cycle progression including neuronal apoptosis, learning process, drug-induced behavorial responses, bone growth and differentiation, and embryo development. For instance, cell transformation by oncogenes that function in the growth factor signal transduction pathway, such as *ras*, *ras*F and *mek*, results in a high increase in AP1 component protein expression. Therefore, AP1-regulated genes support the invasive process observed during malignancy and metastasis.

Platform Microplate reader

Properties

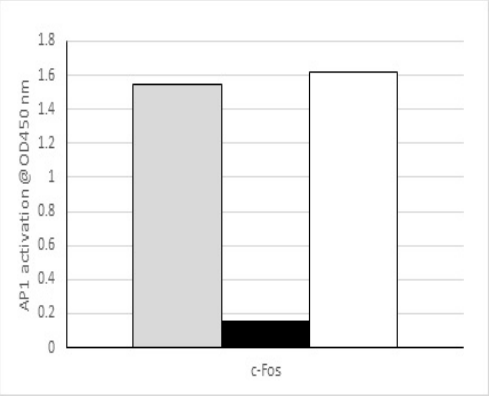
Storage instructions Please refer to protocols.

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 assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	1 x 55µl
AP-1 Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl
AP-1 Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 5µl
Binding Buffer	1 x 10ml	1 x 50ml
c-Fos antibodies	1 x 11µl	1 x 55µl
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
K-562(TPA) nuclear extract (2.5µg/µL)	1 x 40µl	1 x 200µl

Components	1 x 96 tests	5 x 96 tests
Lysis Buffer	1 x 10ml	1 x 50ml
Plate sealer	1 unit	5 units
Poly [d(I-c)] (17 µg/µL)	1 x 100µl	1 x 500µl
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
Stop Solution	1 x 11ml	1 x 55ml

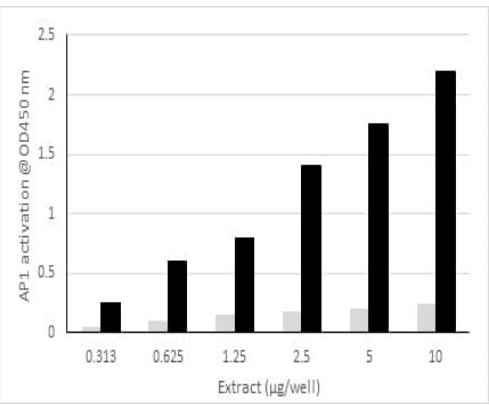
Function	Nuclear phosphoprotein which forms a tight but non-covalently linked complex with the JUN/AP-1 transcription factor. In the heterodimer, FOS and JUN/AP-1 basic regions each seems to interact with symmetrical DNA half sites. On TGF-beta activation, forms a multimeric SMAD3/SMAD4/JUN/FOS complex at the AP1/SMAD-binding site to regulate TGF-beta-mediated signaling. Has a critical function in regulating the development of cells destined to form and maintain the skeleton. It is thought to have an important role in signal transduction, cell proliferation and differentiation.
Sequence similarities	Belongs to the bZIP family. Fos subfamily. Contains 1 bZIP domain.
Post-translational modifications	Phosphorylated in the C-terminal upon stimulation by nerve growth factor (NGF) and epidermal growth factor (EGF). Phosphorylated, in vitro, by MAPK and RSK1. Phosphorylation on both Ser-362 and Ser-374 by MAPK1/2 and RSK1/2 leads to protein stabilization with phosphorylation on Ser-374 being the major site for protein stabilization on NGF stimulation. Phosphorylation on Ser-362 and Ser-374 primes further phosphorylations on Thr-325 and Thr-331 through promoting docking of MAPK to the DEF domain. Phosphorylation on Thr-232, induced by HA-RAS, activates the transcriptional activity and antagonizes sumoylation. Phosphorylation on Ser-362 by RSK2 in osteoblasts contributes to osteoblast transformation. Constitutively sumoylated by SUMO1, SUMO2 and SUMO3. Desumoylated by SENP2. Sumoylation requires heterodimerization with JUN and is enhanced by mitogen stimulation. Sumoylation inhibits the AP-1 transcriptional activity and is, itself, inhibited by Ras-activated phosphorylation on Thr-232.
Cellular localization	Nucleus.

Images



Nuclear extracts from K-562 cells stimulated with TPA were assayed for activity of AP1 family member c-Fos.

Nuclear extracts from K-562 cells stimulated with TPA (Gray) were tested for activity of AP1 family member c-Fos with 5 µg/well of nuclear extract in the absence or presence of wild-type (Black) or mutated (White) consensus binding oligonucleotides. These results are provided for demonstration purposes only.



Different amounts of unstimulated (gray) and PMA/Ionomycin-stimulated (black) WI-38 cells were tested for AP1 activation. These results are provided for demonstration purposes only.

Different amounts of unstimulated (gray) and PMA/Ionomycin-stimulated (black) WI-38 cells were tested for AP1 activation. These results are provided for demonstration purposes only.

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