

Product datasheet

Fos B Transcription Factor Assay Kit (Colorimetric)
ab207197

1 Image

Overview

Product name	Fos B Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	< 1250 ng/ml
Assay time	3h 30m
Species reactivity	Reacts with: Mouse, Rat, Human
Product overview	Fos B Transcription Factor Assay Kit (Colorimetric) (ab207197) is a high throughput assay to quantify AP-1 Fos B 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. AP1 present in the nuclear extract specifically binds to the oligonucleotide. AP1 family member Fos B is detected by a primary antibody that recognizes an epitope of Fos B 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 Fos B.

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. The members of this family, named bZIP, share a dimerization domain with a leucine zipper motif and a DNA binding domain rich in basic residues (lysines and arginines). 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 behavioral 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*, *rasF* 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 (0.25 µg/µL)	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 500µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
FosB antibodies	1 x 11µl	1 x 55µl
K-562(TPA) nuclear extract (2.5µg/µL)	1 x 40µl	1 x 200µl

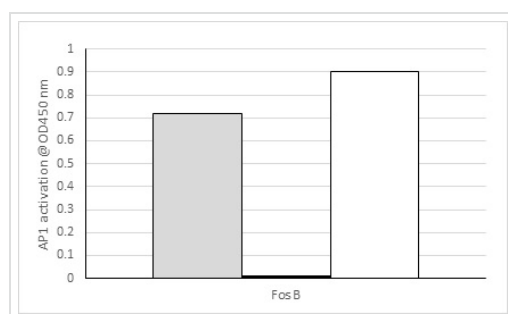
Components	1 x 96 tests	5 x 96 tests
Lysis Buffer	1 x 10ml	1 x 50ml
Plate sealer	1 unit	5 units
Poly [d(l-c)] (17 µg/µL)	1 x 100µl	1 x 500µl
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
Stop Solution	1 x 11ml	1 x 55ml

Function FosB interacts with Jun proteins enhancing their DNA binding activity.

Sequence similarities Belongs to the bZIP family. Fos subfamily.
Contains 1 bZIP domain.

Cellular localization Nucleus.

Images



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

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

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