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Product datasheet

JunD Transcription Factor Assay Kit (Colorimetric) ab207198

1 图像

概述

产品名称	JunD Transcription Factor Assay试剂盒(Colorimetric)
检测方法	Colorimetric
样品类型	Nuclear Extracts
检测类型	Semi-quantitative
灵敏度	< 1250 ng/well
检测时间	3h 30m
种属反应性	**与反应:** Mouse, Rat, Human
产品概述	JunD Transcription Factor Assay Kit (Colorimetric) (ab207198) is a high throughput assay to quantify JunD 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 JunD is detected by a primary antibody that recognizes an epitope of JunD 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 JunD. 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.
说明	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 Jun D. 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. In the case of c-Jun, the activation domain is regulated to a large extent by the JNK family of MAP kinases. JNK kinases phosphorylate c-Jun at Ser-63, resulting in the binding of c-Jun to the CBP/p300 family of transcriptional co-activators. For the Fos proteins, both N- and C-terminal domains flanking the bZIP domain require phosphorylation for biological activity. The kinases responsible for activation remain to be determined.

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. The total increase in AP1 DNA binding activity after any stimulus is often much less impressive than would be expected based on analysis of mRNA expression. This increase is accompanied by a change in the composition of AP1 complexes shortly after stimulation in order to modulate transcriptional control. 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.

平台 Microplate reader

性能

存放说明 Please refer to protocols.

组件	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 500µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml

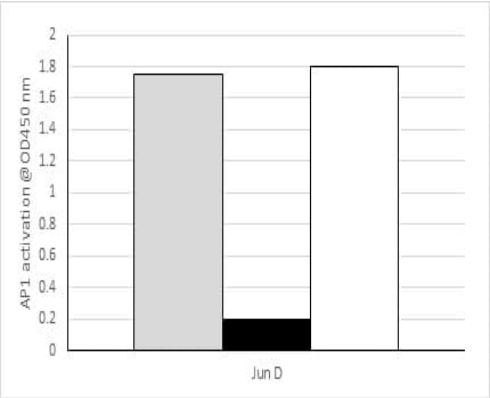
组件	1 x 96 tests	5 x 96 tests
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
JunD antibodies	1 x 11µl	1 x 55µl
K-562(TPA) nuclear extract (2.5µg/µL)	1 x 40µl	1 x 200µl
Lysis Buffer	1 x 10ml	1 x 50ml
Plate sealer	1 unit	5 units
Poly [d(I-c)] (17 µg/µL)	1 x 100µl	1 x 500µl
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
Stop Solution	1 x 11ml	1 x 55ml

功能 Transcription factor binding AP-1 sites.

序列相似性 Belongs to the bZIP family. Jun subfamily.
Contains 1 bZIP domain.

细胞定位 Nucleus.

图片



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

Nuclear extracts from K-562 cells stimulated with TPA (Grey) were assayed for activity of AP1 family member Jun D 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.

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