abcam

Product datasheet

MAPK (ATF2, c-Jun, c-Myc, MEF2, STAT1alpha) Transcription Factor Assay Kit (Colorimetric) ab207212

1 Image

Overview

Product name	MAPK (ATF2, c-Jun, c-Myc, MEF2, STAT1alpha) Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	500 ng/well
Assay time	3h 30m
Species reactivity	**Reacts with:** Mouse, Human
Product overview	MAPK (ATF2, c-Jun, c-Myc, MEF2, STAT1alpha) Transcription Factor Assay Kit (Colorimetric) (ab207212) is a high throughput assay to quantify activation of MAPK regulated transcription factors. 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 MAPK consensus binding site has been immobilized onto a 96-well plate. Active MAPK present in the nuclear extract specifically binds to the oligonucleotide. MAPK is detected by a primary antibody that recognizes an epitope of MAPK 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 MAPK and mice c-Myc, c Jund and Jun D. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 0.5 µg nuclear extract/well. Detection range: 0.5 – 5 µg of nuclear extract/well for c-Myc, MEF2 and STAT1; 1 – 10 µg nuclear extract/well for ATF-2 and c-Jun.
Notes	The transmission of extracellular signals into intracellular responses is a complex process that often involves the activity of mitogen-activated protein kinases (MAPKs). The MAPK pathway is a

three kinase cascade consisting of a MAPK kinase (MAPKKK or MEKK) that activates a MAP/ERK kinase (MEK or MAPKK). This stimulates a phosphorylation-dependent increase in the activity of the MAP kinase. Upon activation, MAPKs phosphorylate a variety of intracellular targets including transcription factors, transcription adaptor proteins, membrane and cytoplasmic substrates as well as other protein kinases.

At least three parallel MAPK pathways exist in humans. The extracellular signal-regulated protein kinase (ERK) pathway primarily transmits mitogenic and differentiation stimuli, while the c-Jun N-terminal kinase (JNK) and p38 pathways predominantly transmit stress and cytokine stimuli. c-Myc, an ERK substrate, is a transcription factor that regulates cell growth and differentiation, glycolysis and apoptosis. Deregulation of c-Myc has been implicated in the origin of diverse human cancers. Elk-1 is a member of the ternary complex factor (TCF) sub-family of the ETS domain family. Elk-1 can be stimulated by all three MAPK pathways, and its main function is the regulation of the activity of the c-Fos promoter in response to extracellular stimuli. MEF2, a member of the MADS box family, is mainly involved in muscle differentiation, but also plays roles in muscle hypertrophy, neuronal survival and T-cell apoptosis. MEF2 is activated by both the p38 and ERK5 pathways.1 STAT1 is involved in activation of IFNα and γ genes, and is activated by p38 and JNK pathways. c-Jun is a member of the activator protein-1 (AP-1) family and is activated by both ERK1/2 and JNK pathways.2 AP-1 members play roles in the expression of genes involved in proliferation and cell cycle progression. ATF-2 is a member of the ATF/CREB family that binds to the cAMP response element (CRE). ATF-2 is activated by ERK1/2, JNK and p38.

Platform Microplate reader

Properties

Storage instructions Please refer to protocols.

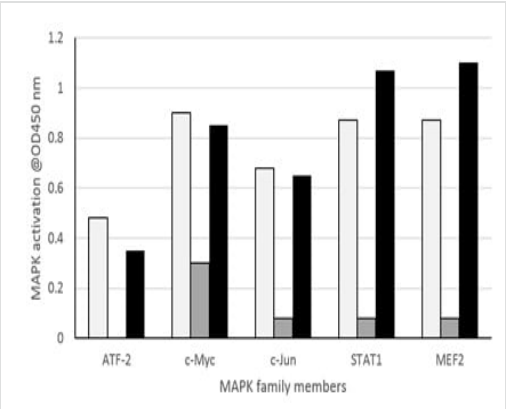
Components	2 x 96 tests
10X Antibody Binding Buffer	2 x 2.2ml
10X Wash Buffer	1 x 60ml
96-well MAPK assay plate	2 units
Anti-mouse HRP-conjugated IgG	1 x 11µl
Anti-rabbit HRP-conjugated IgG (0.25 µg/µL)	2 x 11µl
AP-1 Mutated oligonucleotide (10 pmol/µL)	1 x 100µl
AP-1 Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl
ATF-2 Mutated oligonucleotide (10 pmol/µL)	1 x 100µl
ATF-2 Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl
Binding Buffer	1 x 10ml
c-Myc antibody	1 x 11µl

Components	2 x 96 tests
c-Myc Mutated oligonucleotide (10 pmol/µL)	1 x 100µl
c-Myc Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl
Developing Solution	2 x 11ml
Dithiothreitol (DTT) (1 M)	1 x 100µl
K-562(TPA) nuclear extract (2.5µg/µL)	1 x 40µl
Lysis Buffer	1 x 10ml
MEF2 antibody	1 x 11µl
MEF2 mutated oligonucleotide (10 pmol/µL)	1 x 100µl
MEF2 wild-type oligonucleotide (10 pmol/µL)	1 x 100µl
Phospho-c-Jun antibody	1 x 22µl
Phosphorylated ATF-2 antibody	1 x 11µl
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
Stop Solution	1 x 60ml

Relevance

MAPK is activated by phosphorylation, whereupon the protein translocates to the nucleus, where it phosphorylates nuclear targets. MAPK is an important integration point of many signalling pathways, for a range of cellular processes including proliferation, differentiation, transcription regulation and development.

Images



Example Data

Nuclear extracts from various cell lines were tested for ATF2, c-Myc, c-Jun, STAT1 and MEF2 activation. Cell lines used were HeLa (anisomycin-treated) for ATF2, Jurkat (1 day growth) for c-Myc, K-562 (TPA-treated) for c- Jun, U-937 (TPA + IFNγ treated) for STAT1 and C2C12 for MEF2. Activation was also monitored in the absence (grey) and in the presence of wild-type (black) or mutated (white) consensus binding oligonucleotides. Note that the wild-type oligonucleotide reduces binding of the transcription factors by over 90%, while incubation with the mutant MAPK competitor oligos have limited effect on DNA binding. These results are provided for demonstration purposes only.

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