abcam

Product datasheet

Total CREB Transcription Factor Assay Kit (Colorimetric) ab207201

1 Image

Overview

Product name	Total CREB 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, Rat, Human, Monkey
Product overview	Total CREB Transcription Factor Assay Kit (Colorimetric) (ab207201) is a high throughput assay to quantify total CREB in nuclear extracts. This assay combines a quick ELISA format with a sensitive and specific non-radioactive assay for transcription factor binding. A specific double stranded DNA sequence containing the total CREB consensus binding site (5' –TGACGTCA– 3') has been immobilized onto a 96-well plate. Total CREB present in the nuclear extract specifically binds to the oligonucleotide. Total CREB is detected by a primary antibody that recognizes an epitope of total CREB accessible only when the protein is bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout that at OD 450 nm. This product detects total CREB in human, mouse, rat and monkey samples. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 0.5 µg nuclear extract/well. Detection range: 0.3 – 10 µg nuclear extract/well.
Notes	CREB (Cyclic AMP Response Element-Binding protein) is a member of a large family of structurally related transcription factors that includes AFT1-4, 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). CREB proteins specifically

recognize the cAMP-responsive element promoter site.

Alternative splicing of the CREB gene yields several forms of CREB protein. The three most abundant forms are CREBα (341 aa), also called CREB1, CREBΔ (327 aa) and CREBβ (301 and 387 aa), which are present in human, rat and mouse tissues. Two other gene products highly homologous to CREB-1 have been characterized: activating transcription factor 1 (ATF-1) and cAMP response element modulator (CREM).

The CREB family members bind to the CRE promoter site as homo- and heterodimers. The ratio between these homo- and heterodimers, which depends on the cell type, regulates the CREB transcriptional activity as the homodimers have a longer half-life than CREB/ATF-1 heterodimers.

The CREB proteins activate transcription of target genes in response to a diverse array of stimuli, such as peptide hormones, growth factors and neuronal activity. Activation of CREB is mediated by a variety of protein kinases including protein kinase A (PKA), mitogen-activated protein kinases (MAPKs) and Ca^{2+}/calmodulin-dependent protein kinases (CaMKs) that phosphorylate CREB at the Ser-133 residue. Phosphorylation of Ser 133 is required for CREB-mediated transcription but not for dimerization and DNA-binding activity. Phosphorylation does, however, increase CREB's affinity for its promoter site. Upon cell stimulation with forskolin, an activator of adenylyl cyclase, the kinases are activated by cAMP production and translocate to the nucleus where they phosphorylate CREB at Ser 133. Therefore, CREB is almost exclusively nuclear in both unstimulated and stimulated cells. A cofactor, CREB-binding protein (CPB), specifically binds to phosphorylated CREB to enhance transcriptional activity.

Platform Microplate reader

Properties

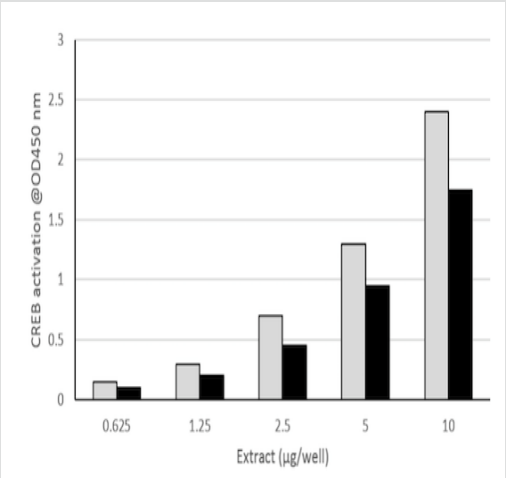
Storage instructions Please refer to protocols.

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 CREB assay plate	1 unit	5 units
Anti-mouse HRP-conjugated IgG	1 x 11µl	1 x 55µl
Binding Buffer	1 x 10ml	1 x 50ml
CREB antibody	1 x 10µl	1 x 50µl
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
Herring sperm DNA	1 x 100µl	1 x 500µl
Lysis Buffer	1 x 10ml	1 x 50ml
ATF-2 Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl

Components	1 x 96 tests	5 x 96 tests
Plate sealer	1 unit	5 units
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
Stop Solution	1 x 11ml	1 x 55ml
WI-38 (Forskolin) nuclear extract (2.5 µg/µL)	1 x 40µl	1 x 200µl
ATF-2 Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl

Function	This protein binds the cAMP response element (CRE), a sequence present in many viral and cellular promoters. CREB stimulates transcription on binding to the CRE. Transcription activation is enhanced by the TORC coactivators which act independently of Ser-133 phosphorylation. Implicated in synchronization of circadian rhythmicity.
Involvement in disease	Defects in CREB1 may be a cause of angiomatoid fibrous histiocytoma (AFH) [MIM:612160]. A distinct variant of malignant fibrous histiocytoma that typically occurs in children and adolescents and is manifest by nodular subcutaneous growth. Characteristic microscopic features include lobulated sheets of histiocyte-like cells intimately associated with areas of hemorrhage and cystic pseudovascular spaces, as well as a striking cuffing of inflammatory cells, mimicking a lymph node metastasis. Note=A chromosomal aberration involving CREB1 is found in a patient with angiomatoid fibrous histiocytoma. Translocation t(2;22)(q33;q12) with CREB1 generates a EWSR1/CREB1 fusion gene that is most common genetic abnormality in this tumor type.
Sequence similarities	Belongs to the bZIP family. Contains 1 bZIP domain. Contains 1 KID (kinase-inducible) domain.
Post-translational modifications	Stimulated by phosphorylation. Phosphorylation of both Ser-133 and Ser-142 in the SCN regulates the activity of CREB and participates in circadian rhythm generation. Phosphorylation of Ser-133 allows CREBBP binding (By similarity). Phosphorylated upon DNA damage, probably by ATM or ATR. Sumoylated by SUMO1. Sumoylation on Lys-304, but not on Lys-285, is required for nuclear localization of this protein. Sumoylation is enhanced under hypoxia, promoting nuclear localization and stabilization.
Cellular localization	Nucleus.

Images



Nuclear extracts from WI-38 cells (Gray) and WI-38 cells. Stimulated with Forskolin (Black) were assayed from 0.625 - 10µg/well for CREB activation using the Total CREB Transcription Factor Assay Kit

Nuclear extracts from WI-38 cells (grey) and WI-38 cells stimulated with Forskolin (black) were tested for CREB activation. These results are provided for demonstration purposes only.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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