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Product datasheet

Nrf2 Transcription Factor Assay Kit (Colorimetric) ab207223

30 References 1 Image

Overview

Product name	Nrf2 Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	> 600 ng/well
Assay time	3h 30m
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	Nrf2 Transcription Factor Assay Kit (Colorimetric) (ab207223) is a high throughput assay to quantify Nrf2 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 Nrf2 consensus binding site (5' – GTCACAGTGACTCAGCAGAATCTG – 3') has been immobilized onto a 96-well plate. Active Nrf2 present in the nuclear extract specifically binds to the oligonucleotide. Nrf2 is detected by a primary antibody that recognizes an epitope of Nrf2 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 only human, mouse and rat Nrf2. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 0.6 µg nuclear extract/well. Detection range: 0.6 – 10 µg nuclear extract/well.
Notes	Nrf2 (NF-E2 related factor, NFE2L2, from nuclear factor erythroid-derived 2-like 2) is a basic leucine zipper (bZIP) transcription factor. Nrf2 binds to the antioxidant response element (ARE) and positively regulates the expression of detoxifying enzyme genes (such as NAD(P)H:quinone oxidoreductase1, NQO1) in response to antioxidants and xenobiotics. Higher levels of NQO1 gene expression has been shown in liver, lung, colon, and breast tumors.

A cytosolic inhibitor of Nrf2, Keap1/INrf2, retains Nrf2 in the cytoplasm under normal conditions where the interaction of Nrf2 with INrf2 targets Nrf2 for ubiquitination and proteasomal degradation. However, after oxidative stress, Nrf2 is released from INrf2, translocates to the nucleus, and results in the activation of ARE-mediated gene expression. Nrf2 is also synthesized *de novo* after exposure to stress. In addition, Nrf2 controls its own degradation by regulating expression and induction of INrf2.

It has been shown that nuclear export and degradation pathways are activated by around two hours after treatment with tert-butylhydroquinone (t-BHQ).

Nrf2 activation and degradation are important sensing mechanisms in the cellular response for oxidative and electrophilic stressors.

Platform Microplate reader

Properties

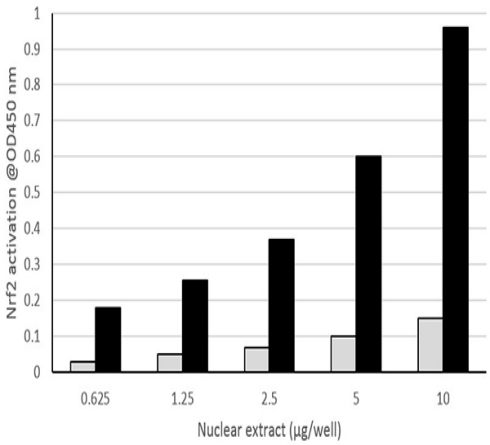
Storage instructions Please refer to protocols.

Components	1 x 96 tests	5 x 96 tests
10X Antibody Binding Buffer	1 x 2.2ml	5 x 2.2ml
10X Wash Buffer	1 x 22ml	5 x 22ml
96-well Nrf2 assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 10µl	5 x 10µl
Binding Buffer	1 x 10ml	5 x 10ml
Developing Solution	1 x 11ml	5 x 11ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	5 x 100µl
Herring sperm DNA	1 x 100µl	5 x 100µl
Lysis Buffer	1 x 10ml	5 x 10ml
Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl
Nrf2 antibody	1 x 10µl	1 x 25µl
Plate sealer	1 unit	5 units
Positive control extract (2.5 µg/µL)	1 x 20µl	1 x 50µl
Protease Inhibitor Cocktail	1 x 100µl	5 x 100µl
Stop Solution	1 x 11ml	5 x 11ml
Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl

Function Transcription activator that binds to antioxidant response (ARE) elements in the promoter regions

	of target genes. Important for the coordinated up-regulation of genes in response to oxidative stress. May be involved in the transcriptional activation of genes of the beta-globin cluster by mediating enhancer activity of hypersensitive site 2 of the beta-globin locus control region.
Tissue specificity	Widely expressed. Highest expression in adult muscle, kidney, lung, liver and in fetal muscle.
Sequence similarities	Belongs to the bZIP family. CNC subfamily. Contains 1 bZIP domain.
Domain	Acidic activation domain in the N-terminus, and DNA binding domain in the C-terminus.
Post-translational modifications	Phosphorylation of Ser-40 by PKC in response to oxidative stress dissociates NFE2L2 from its cytoplasmic inhibitor KEAP1, promoting its translocation into the nucleus.
Cellular localization	Cytoplasm > cytosol. Nucleus. Cytosolic under unstressed conditions, translocates into the nucleus upon induction by electrophilic agents.

Images



Nuclear extracts from untreated HepG2 cells (Light gray) and HepG2 cells treated with D,L Sulforaphane (Black) were assayed from 0.625 to 10 µg/well for Nrf2 activation using ab207223.

Different amounts of nuclear extracts from untreated HepG2 cells (light grey) and HepG2 cells treated with D,L-Sulforaphane (Black) were tested for Nrf2 activation. These results are provided for demonstration only.

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