

NFkB p52 Transcription Factor Assay Kit (Chemiluminescent) ab207220

Overview

Product name	NFkB p52 Transcription Factor Assay Kit (Chemiluminescent)
Detection method	Luminescent
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	< 40 ng/well
Assay time	3h 30m
Species reactivity	Reacts with: Mouse, Human
Product overview	NFkB p52 Transcription Factor Assay Kit (Chemiluminescent) (ab207220) is a high throughput assay to quantify NFkB p52 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 NFkB p52 consensus binding site (5' - GGGACTTTCC - 3') has been immobilized onto a 96-well plate. Active NFkB p52 present in nuclear cell extracts specifically binds to the oligonucleotide. NFkB p52 is detected by a primary antibody that recognizes an epitope of NFkB p52 accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides a sensitive chemiluminescent readout that can be quantified using a luminometer or CCD camera system. This product detects only human and mouse NFkB p52.

Key performance and benefits:

Assay time: 3.5 hours (cell extracts preparation not included).

Detection limit: < 40 ng nuclear extract/well.

Detection range: 0.039 – 2.5 µg nuclear cell extract/well.

Notes

The transcription factor NFkB is implicated in the regulation of many genes that code for mediators of the immune, acute phase and inflammatory responses. NFkB is composed of homo- and heterodimeric complexes of members of the Rel (NFkB) family. There are five subunits of the NFkB family in mammals: p50, p65 (RelA), c-Rel, p52 and RelB. These proteins share a

conserved 300 amino acid sequence in the N-terminal region, known as the Rel homology domain, that mediates DNA binding, protein dimerization and nuclear localization. Various dimer combinations of the NFkB subunits have distinct DNA binding specificities and may serve to activate specific sets of genes such as adhesion molecules, immunoreceptors and cytokines. Proteolytic cleavage of p102 generates the mature NFkB p52 subunit. The p52 homodimers are, in general, repressors of kB site transcription, but they also bind to the nuclear protein Bcl3, and such complexes can function as transcriptional activators.

In the majority of cells, NFkB exists in an inactive form in the cytoplasm, bound to the inhibitory Ikb proteins. Treatment of cells with various inducers results in the phosphorylation, ubiquitination and subsequent degradation of Ikb proteins (For studying the phosphorylation state of Ikb α).

Proteolytic cleavage of p105 results in two proteins: p50, which has DNA-binding activity but no transactivation domain, and its antagonist, the inhibitory Ikb β protein. This results in the release of NFkB dimers, which subsequently translocate to the nucleus, where they activate appropriate target genes. NFkB can be activated by a number of stimuli, including components of bacterial cell walls, such as lipopolysaccharide, or inflammatory cytokines, such as TNF- α or IL-1 β .

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 NFkB chemi assay plate	1 x 96 tests	5 x 96 tests
Anti-rabbit HRP-conjugated IgG	1 x 11 μ l	1 x 55 μ l
Binding Buffer	1 x 10ml	1 x 50ml
Chemiluminescent Reagent	1 x 2ml	1 x 10ml
Dithiothreitol (DTT) (1 M)	1 x 100 μ l	1 x 500 μ l
Herring sperm DNA	1 x 100 μ l	1 x 500 μ l
Lysis Buffer	1 x 10ml	1 x 50ml
Mutated oligonucleotide (10 pmol/ μ L)	1 x 100 μ l	1 x 500 μ l
NFkB p52 antibodies	1 x 11 μ l	1 x 55 μ l
Plate sealer	1 unit	5 units
Protease Inhibitor Cocktail	1 x 100 μ l	1 x 500 μ l
Raji nuclear extract (2.5 μ g/ μ L)	1 x 40 μ l	1 x 200 μ l

Components	1 x 96 tests	5 x 96 tests
Reaction Buffer	1 x 4ml	1 x 20ml
Wild-type oligonucleotide (10 pmol/μL)	1 x 100μl	1 x 500μl

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

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