

c-Myc Transcription Factor Assay Kit (Colorimetric) ab207200

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Overview

Product name	c-Myc Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	< 250 ng/well
Assay time	3h 30m
Species reactivity	Reacts with: Mouse, Human
Product overview	<p>c-Myc Transcription Factor Assay Kit (Colorimetric) (ab207200) is a high throughput assay to quantify c-Myc activation in nuclear extracts. This assay combines a quick ELISA format with a sensitive and specific non-radioactive assay for transcription factor activation.</p> <p>A specific double stranded DNA sequence containing the c-Myc consensus binding site (5' – CACGTG– 3') has been immobilized onto a 96-well plate. Active c-Myc present in the nuclear extract specifically binds to the oligonucleotide. c-Myc is detected by a primary antibody that recognizes an epitope of c-Myc accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout that at OD 450 nm. This product detects human and mouse c-Myc.</p> <p>Key performance and benefits:</p> <p>Assay time: 3.5 hours (cell extracts preparation not included).</p> <p>Detection limit: < 0.25 µg nuclear extract/well.</p> <p>Detection range: 0.25 – 5 µg nuclear extract/well.</p>
Notes	<p>c-Myc is a transcription factor that regulates cell growth and differentiation, glycolysis and apoptosis and deregulation of c-Myc has been implicated in the origin of diverse human cancers.</p> <p>c-Myc was originally discovered as the cellular homolog of the retroviral v-myc oncogene, and is a transcription factor involved in a wide variety of cellular processes, including cell proliferation,</p>

replicative potential, growth, differentiation and apoptosis. Expression of c-Myc is induced by mitogenic signals and suppressed by growth-inhibitory signals. c-Myc is a member of the basic helix-loop-helix leucine zipper (bHLHzip) family, along with B-Myc, N-Myc, L-Myc and s-Myc. Upon dimerization with the bHLHzip protein Max, c-Myc can bind to the E box motif CACGTG and activate transcription. c-Myc is phosphorylated at Ser62, which has been shown to be a regulatory site of phosphorylation. The phosphorylation of c-Myc causes increased function of the NH₂-terminal transactivation domain, and studies have indicated that the expression of MAP kinase is responsible for increased c-Myc phosphorylation at Ser62.

Because of the central role of c-Myc as an activator of diverse cellular processes, regulation of this transcription factor is crucial for proper cell function and ultimate survival. The main regulation of c-Myc occurs through its binding with the bHLHzip protein Max, which can also form heterodimers with members of the Mad family (Mad 1, 3, 4 and Mxi1). As c-Myc cannot bind to DNA without forming a heterodimer with Max, competition between c-Myc and Mad for the common partner Max is used to regulate c-Myc activity. While Max is a relatively stable protein, c-Myc degrades rapidly, with a half-life of 20-30 minutes.

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 c-Myc assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	5 x 11µl
Binding Buffer	1 x 10ml	5 x 10ml
c-Myc antibody	1 x 11µl	5 x 11µl
c-Myc Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl
c-Myc Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl
Developing Solution	1 x 11ml	5 x 11ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	5 x 100µl
Jurkat (1 day growth) nuclear extract	1 x 40µl	5 x 40µl
Lysis Buffer	1 x 10ml	5 x 10ml
Plate sealer	1 unit	5 units
Protease Inhibitor Cocktail	1 x 100µl	5 x 100µl

Components	1 x 96 tests	5 x 96 tests
Stop Solution	1 x 11ml	5 x 11ml

Function

Participates in the regulation of gene transcription. Binds DNA in a non-specific manner, yet also specifically recognizes the core sequence 5'-CAC[GA]TG-3'. Seems to activate the transcription of growth-related genes.

Involvement in disease

Note=Overexpression of MYC is implicated in the etiology of a variety of hematopoietic tumors. Note=A chromosomal aberration involving MYC may be a cause of a form of B-cell chronic lymphocytic leukemia. Translocation t(8;12)(q24;q22) with BTG1.

Defects in MYC are a cause of Burkitt lymphoma (BL) [MIM:113970]. A form of undifferentiated malignant lymphoma commonly manifested as a large osteolytic lesion in the jaw or as an abdominal mass. Note=Chromosomal aberrations involving MYC are usually found in Burkitt lymphoma. Translocations t(8;14), t(8;22) or t(2;8) which juxtapose MYC to one of the heavy or light chain immunoglobulin gene loci.

Sequence similarities

Contains 1 basic helix-loop-helix (bHLH) domain.

Post-translational modifications

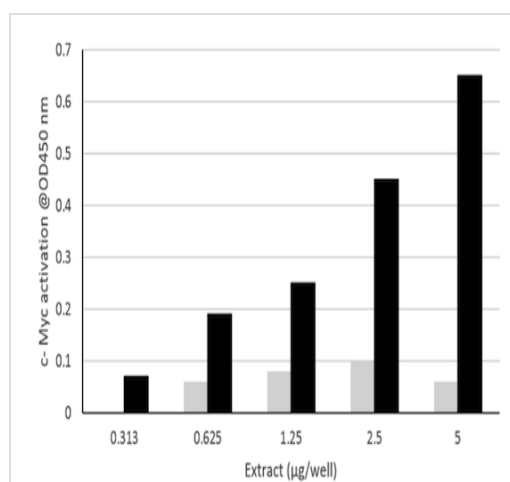
Phosphorylated by PRKDC. Phosphorylation at Thr-58 and Ser-62 by GSK3 is required for ubiquitination and degradation by the proteasome.

Ubiquitinated by the SCF(FBXW7) complex when phosphorylated at Thr-58 and Ser-62, leading to its degradation by the proteasome. In the nucleoplasm, ubiquitination is counteracted by USP28, which interacts with isoform 1 of FBXW7 (FBW7alpha), leading to its deubiquitination and preventing degradation. In the nucleolus, however, ubiquitination is not counteracted by USP28, due to the lack of interaction between isoform 4 of FBXW7 (FBW7gamma) and USP28, explaining the selective MYC degradation in the nucleolus. Also polyubiquitinated by the DCX(TRUSS) complex.

Cellular localization

Nucleus > nucleoplasm. Nucleus > nucleolus.

Images



Different amounts of unstimulated U-937 (grey) and Jurkat (black) were tested for c-Myc activation. These results are provided for demonstration purposes only.

Different amounts of unstimulated Jurkat (Gray) and U-937 (Black) were tested for c-Myc activation.

These results are provided for demonstration purposes only.

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