abcam

Product datasheet

Glucocorticoid Receptor Transcription Factor Assay Kit (Colorimetric) ab207207

1 References 1 Image

Overview

Product name	Glucocorticoid Receptor 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	Glucocorticoid Receptor Transcription Factor Assay Kit (Colorimetric) (ab207207) is a high throughput assay to quantify Glucocorticoid Receptor (GR) 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 Glucocorticoid Receptor consensus binding site (5' –GGTACAnnnTGTTCT– 3') has been immobilized onto a 96-well plate. Active Glucocorticoid Receptor present in the nuclear extract specifically binds to the oligonucleotide. Glucocorticoid Receptor is detected by a primary antibody that recognizes an epitope of Glucocorticoid Receptor 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, mouse and rat Glucocorticoid Receptor. 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	Glucocorticoids can affect a large number of metabolic, cardiovascular, immune, inflammatory and behavioral functions. They are produced by the adrenal cortex and are under the control of the

hypothalamus and pituitary (hypothalamus-pituitary-adrenal [HPA] axis). At the cellular level, glucocorticoid effects are mediated by the Glucocorticoid Receptor (GR). GR belongs to the superfamily of nuclear hormone receptors that includes receptors for estrogens, progestins, vitamin D and thyroid hormone.

The nuclear hormone receptors share a characteristic three-domain structure. The N-terminal activates target genes and interacts with transcription machinery. Two highly conserved zinc fingers constitute the DNA-binding domain and also participate in dimerization, nuclear translocation and transactivation. The C-terminal contains the ligand-binding domain, and also includes sequences important for heat shock protein (hsp) binding, nuclear translocation, dimerization and transactivation.

The unliganded GR is part of a multiprotein complex that consists of the receptor, two molecules of hsp90 and one molecule each of hsp70 and hsp56. Glucocorticoids, when present, are able to cross the cell membrane and interact with GR. When bound, there is a conformational change in the GR molecule that results in dissociation from the hsp complex, hyper-phosphorylation of GR and unmasking of nuclear localization signals. When in the nucleus, the activated GR can act in two ways: directly with specific DNA sequences or indirectly with other transcription factors. GR mutations can result in glucocorticoid resistance or hypersensitivity, and can cause severe disturbances in mood, pathologic alterations of metabolism and, correspondingly, hypotension or hypertension and excessive or suppressed inflammatory/immune responses.

Platform Microplate reader

Properties

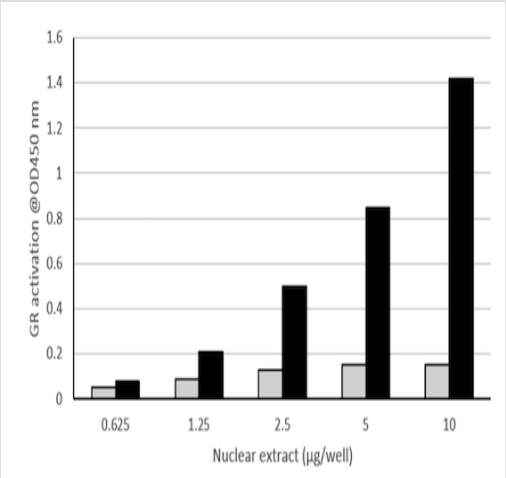
Storage instructions Please refer to protocols.

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 GR assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	1 x 55µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
GR antibody	1 x 11µl	1 x 55µl
HeLa nuclear extract	1 x 40µl	1 x 200µl
Herring sperm DNA	1 x 100µl	1 x 500µl
Lysis Buffer	1 x 10ml	1 x 50ml
Mutated oligonucleotide(20 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
Wild-type oligonucleotide (20 pmol/µL)	1 x 100µl	1 x 500µl

Function	Receptor for glucocorticoids (GC). Has a dual mode of action: as a transcription factor that binds to glucocorticoid response elements (GRE) and as a modulator of other transcription factors. Affects inflammatory responses, cellular proliferation and differentiation in target tissues. Could act as a coactivator for STAT5-dependent transcription upon growth hormone (GH) stimulation and could reveal an essential role of hepatic GR in the control of body growth. Involved in chromatin remodeling. Plays a significant role in transactivation. Involved in nuclear translocation.
Tissue specificity	Widely expressed. In the heart, detected in left and right atria, left and right ventricles, aorta, apex, intraventricular septum, and atrioventricular node as well as whole adult and fetal heart.
Involvement in disease	Defects in NR3C1 are a cause of glucocorticoid resistance (GCRES) [MIM:138040]; also known as cortisol resistance. It is a hypertensive, hyperandrogenic disorder characterized by increased serum cortisol concentrations. Inheritance is autosomal dominant.
Sequence similarities	Belongs to the nuclear hormone receptor family. NR3 subfamily. Contains 1 nuclear receptor DNA-binding domain.
Domain	Composed of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.
Post-translational modifications	Increased proteasome-mediated degradation in response to glucocorticoids. Phosphorylated in the absence of hormone; becomes hyperphosphorylated in the presence of glucocorticoid. The Ser-203-phosphorylated form is mainly cytoplasmic, and the Ser-211-phosphorylated form is nuclear. Transcriptional activity correlates with the amount of phosphorylation at Ser-211. Sumoylated; this reduces transcription transactivation. Ubiquitinated; restricts glucocorticoid-mediated transcriptional signaling.
Cellular localization	Cytoplasm. Nucleus. Cytoplasmic in the absence of ligand, nuclear after ligand-binding and Nucleus. Localized largely in the nucleus.

Images



Different amounts of nuclear extracts from untreated (Gray) and Dexamethasone-treated (Black) HeLa cells are tested for GR activity. These results are provided for demonstration purposes only.

Different amounts of nuclear extracts from untreated (grey) and Dexamethasone-treated (black) HeLa cells were tested for GR activity. These results are provided for demonstration purposes only.

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