abcam

Product datasheet

Estrogen Receptor Transcription Factor Assay Kit (Colorimetric) ab207203

1 Image

Overview

Product name	Estrogen 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	Estrogen Receptor Transcription Factor Assay Kit (Colorimetric) (ab207203) is a high throughput assay to quantify Estrogen Receptor (ER) 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 Estrogen Receptor consensus binding site (5' –GGTCACAGTGACC– 3') has been immobilized onto a 96-well plate. Active Estrogen Receptor present in the nuclear extract specifically binds to the oligonucleotide. Estrogen Receptor is detected by a primary antibody that recognizes an epitope of Estrogen 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 Estrogen 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 – 20 µg nuclear extract/well.
Notes	ER belongs to the superfamily of ligand-inducible transcription factors. Human ERa is comprised of 595 amino acids and displays an approximate molecular weight of 66-70 kDa. Six functional regions have been identified. A hypervariable domain (aa 1-184) contains activation function 1

(AF1). The DNA binding domain (DBD, aa 185-263) contains two zinc finger motifs and is highly conserved across the nuclear receptor superfamily. It is responsible for the binding of the receptor to estrogen response elements (EREs) and contributes to dimerization and activation. Typically, EREs consist of two inverted half-sites separated by 3 bp (5´-GGTCAnnnTGACC-3´). The region which separates the ligand binding domain (LBD) and the DBD is called the hinge region (aa 264-302).

The LBD (aa 303-553) consists of 12 a-helices, which form a hydrophobic pocket responsible for ligand binding. The function of the final domain (aa 554-595) is not clear but is thought to play a role in distinguishing between agonist and antagonist binding. Human ERb is expressed as multiple isoforms. Structure and function studies have shown that the DBD of ERa and ERb are highly homologous, approaching 96%, whereas the LBD showed only 59% homology. The general mechanism of action of ERb is thought to be similar to that of ERa. ERa and ERb have the ability to interact with target promoters in three different complexes: ERa homodimers, ERb homodimers and ERa/ERb heterodimers.

The transcriptional effects of ER can be mediated through several mechanisms other than E_2-ER complexes binding to EREs. E_2-ER complexes can also trans activate genes through protein-protein interactions with transcription factors such as AP-1 or Sp1 that bind DNA, with coaccessory proteins (Src, ACTR), some of which have histone acetylase activity, and with RNA Polymerase II complex proteins. In addition, ERs serve to repress genes, which also plays an important role in E_2 action.

Platform Microplate reader

Properties

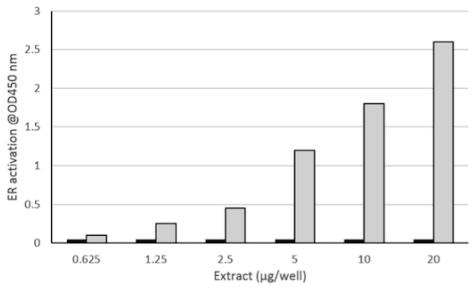
Storage instructions Please refer to protocols.

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 ER assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG (0.25 µg/µL)	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
ERa antibody	1 x 11µl	1 x 55µl
Lysis Buffer	1 x 10ml	1 x 50ml
MCF-7 nuclear extract (2.5 µg/µL)	1 x 40µl	1 x 200µl
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
Poly [d(I-c)] (17 µg/µL)	1 x 100µl	1 x 500µl
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl
Stop Solution	1 x 11ml	1 x 55ml
Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl

Function	Nuclear hormone receptor. The steroid hormones and their receptors are involved in the regulation of eukaryotic gene expression and affect cellular proliferation and differentiation in target tissues. Can activate the transcriptional activity of TFF1.
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	Phosphorylated by cyclin A/CDK2. Phosphorylation probably enhances transcriptional activity. Glycosylated; contains N-acetylglucosamine, probably O-linked. Ubiquitinated. Deubiquitinated by OTUB1. Dimethylated by PRMT1 at Arg-260. The methylation may favor cytoplasmic localization. Palmitoylated (isoform 3). Not biotinylated (isoform 3).
Cellular localization	Nucleus. Cytoplasm. Cell membrane. A minor fraction is associated with the inner membrane and Nucleus. Cytoplasm. Cell membrane. Associated with the inner membrane via palmitoylation.

Images



Different amounts of untreated (Gray) and H2O2 (Black) post-treated nuclear extracts from MCF-7 cells are tested for ERa activity.

Different amounts of untreated (grey) and hydrogen peroxide treated (black) MCF-7 were tested for ERα activity. These results are provided for demonstration purposes only.

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