

IRF3 Transcription Factor Assay Kit (Colorimetric) ab207210

2 References 1 图像

概述

产品名称	IRF3 Transcription Factor Assay试剂盒(Colorimetric)
检测方法	Colorimetric
样品类型	Nuclear Extracts
检测类型	Semi-quantitative
灵敏度	500 ng/well
检测时间	3h 30m
种属反应性	与反应: Human
产品概述	<p>IRF3 Transcription Factor Assay Kit (Colorimetric) (ab207210) is a high throughput assay to quantify IRF3 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 IRF3 consensus binding site (5' – GAAACTGAAACT – 3') has been immobilized onto a 96-well plate. Active IRF3 present in the nuclear extract specifically binds to the oligonucleotide. IRF3 is detected by a primary antibody that recognizes an epitope of IRF3 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 IRF3.</p> <p>Key performance and benefits:</p> <p>Assay time: 3.5 hours (cell extracts preparation not included).</p> <p>Detection limit: < 0.5 µg nuclear extract/well.</p> <p>Detection range: 0.5 – 10 µg nuclear extract/well.</p>

说明

The interferon (IFN) regulatory factor (IRF) family is a group of transcription factors that have extensive homology in their DNA-binding domain (DBD). The many members of the IRF family are involved in the regulation of interferon (IFN) a and b and play a role in host anti-viral immune regulation, cell growth and hematopoietic development. The N-terminal binding domain of IRFs, the distinct feature of the family, is a modified helix-turn-helix characterized by repeated tryptophan residues separated by 10 to 18 amino acids. All IRFs, except IRF1 and IRF2, have an

IRF association domain (IAD) that is responsible for the interaction with other family members or with transcription factors such as PU.1, E47 and STAT. Another association domain (IAD2), present only in IRF1 and IRF2, is important for interaction with IRF8. A nuclear localization signal has been identified in IRF1, and similar sequences may also be present in other family members. A bipartite nuclear retention signal located within the N-terminus of the DBD has been identified in IRF4, IRF8 and IRF9. IRFs also possess a transactivation domain in the middle of the protein.

IRFs bind DNA as dimers on sequences such as the IFN-stimulated response element (ISRE), AGTTTCNNCNY, the IFN consensus sequence (ICS), R(G/C)TTTC, or the IFN-regulatory factor element (IRFE), G(A)AAA(G/C)YGAAA(G/C)Y. While IRF3 is constitutively expressed, the expression of other IRFs is induced by such stimuli as type I and II IFN, double-stranded RNA or the presence of viral components, which can also induce the activity of the IRF factors after they have been synthesized. IRF factors can cooperate with other factors with neighboring binding sites on promoters. For example, the IFN- β promoter provides the stepping stone for the formation of an “enhanceosome” containing ATF-2, c-Jun, IRF3, NF κ B and CBP.

In mammals, ten IRF family members have been identified. IRF1, an activator, is involved in mature lymphocyte apoptosis after DNA-damage. IRF2 is mainly a repressor, but can also play a role in histone H4 expression. Both IRF1 and IRF2 interact with the co-activator P/CAF. IRF1 has been described as a tumor suppressor and IRF2 as a proto-oncogene. IRF3 is a component of DRAAF, a complex containing the acetylase CBP/p300, which binds ISRE-like sequences. Double-stranded RNA (dsRNA) or poly (I-C), a synthetic form of dsRNA, elicits IRF3 activation. IRF4 is required for the function and homeostasis of B and T-cells and can also interact with the Ets-family member PU.1 and with the E47 form of E2A. IRF7 helps induce the IFN- α gene and repress the EBNA-1 gene from the Epstein-Barr virus. The expression of IRF7 is induced by type I IFN, which is important for amplification of the signaling pathway. IRF8 (ICSBP) can interact with IRF1 and 2, but primarily acts as a repressor. IRF9 (ISGF3g/p48) is part of the ISGF3 transcription factor, together with STAT1 and STAT2. IRF10 functions in the late stages of anti-viral defense by regulating IFN γ target genes.

平台 Microplate reader

性能

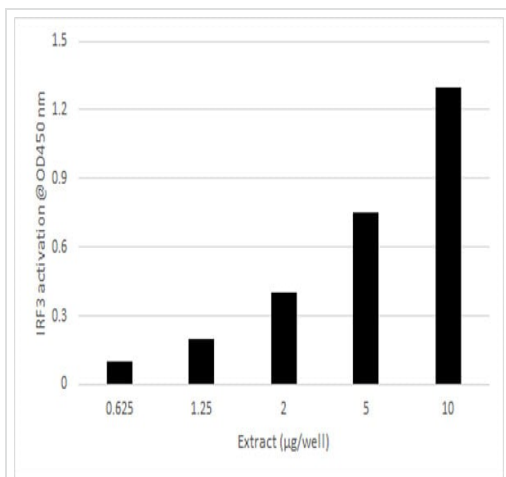
存放说明 Please refer to protocols.

组件	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 IRF-3 assay plate	1 unit	5 units
Anti-mouse HRP-conjugated IgG	1 x 10 μ l	5 x 10 μ l
Binding Buffer	1 x 10ml	5 x 10ml
Cos-7 nuclear extract (Poly (I-C) 2 hr) (2.5 μ g/ μ L)	1 x 40 μ l	5 x 40 μ l
Developing Solution	1 x 11ml	5 x 11ml
Dithiothreitol (DTT) (1 M)	1 x 100 μ l	5 x 100 μ l

组件	1 x 96 tests	5 x 96 tests
IRF-3 antibody (human)	1 x 10µl	1 x 25µl
Lysis Buffer	1 x 10ml	5 x 10ml
Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl
Plate sealer	1 unit	5 units
Poly [d(l-c)] (17 µg/µL)	1 x 100µl	5 x 100µl
Protease Inhibitor Cocktail	1 x 100µl	5 x 100µl
STAT Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	5 x 100µl
Stop Solution	1 x 11ml	5 x 11ml

功能	Mediates interferon-stimulated response element (ISRE) promoter activation. Functions as a molecular switch for antiviral activity. DsRNA generated during the course of an viral infection leads to IRF3 phosphorylation on the C-terminal serine/threonine cluster. This induces a conformational change, leading to its dimerization, nuclear localization and association with CREB binding protein (CREBBP) to form dsRNA-activated factor 1 (DRAF1), a complex which activates the transcription of genes under the control of ISRE. The complex binds to the IE and PRDIII regions on the IFN-alpha and IFN-beta promoters respectively. IRF-3 does not have any transcription activation domains.
组织特异性	Expressed constitutively in a variety of tissues.
序列相似性	Belongs to the IRF family. Contains 1 IRF tryptophan pentad repeat DNA-binding domain.
翻译后修饰	Constitutively phosphorylated on many serines residues. C-terminal serine/threonine cluster is phosphorylated in response of induction by IKBKE and TBK1. Ser-385 and Ser-386 may be specifically phosphorylated in response to induction. An alternate model propose that the five serine/threonine residues between 396 and 405 are phosphorylated in response to a viral infection. Phosphorylation, and subsequent activation of IRF3 is inhibited by vaccinia virus protein E3. Ubiquitinated; ubiquitination involves RBCK1 leading to proteasomal degradation. Polyubiquitinated; ubiquitination involves TRIM21 leading to proteasomal degradation. ISGylated by HERC5 resulting in sustained IRF3 activation and in the inhibition of IRF3 ubiquitination by disrupting PIN1 binding. The phosphorylation state of IRF3 does not alter ISGylation.
细胞定位	Cytoplasm. Nucleus. Shuttles between cytoplasmic and nuclear compartments, with export being the prevailing effect. When activated, IRF3 interaction with CREBBP prevents its export to the cytoplasm.

图片



Different amounts of poly (I/C) treated COS7 cells were tested for IRF3 activation. These results are provided for demonstration purposes only.

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