PPAR gamma Transcription Factor Assay Kit ab133101

Overview

Product name: PPAR gamma Transcription Factor Assay Kit
Detection method: Colorimetric
Sample type: Adherent cells, Suspension cells, Nuclear Extracts
Assay type: Semi-quantitative
Species reactivity: Reacts with: Mouse, Rat, Human
Predicted to work with: Mammals

Product overview:
PPAR gamma Transcription Factor Assay kit ab133101 is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts.

A 96 well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the peroxisome proliferator response element (PPRE) is immobilized onto the bottom of wells of a 96 well plate. PPARs contained in a nuclear extract bind specifically to the PPRE. PPAR gamma is detected by addition of specific primary antibody directed against PPAR gamma. A secondary antibody conjugated to HRP is added to provide a sensitive colorometric readout at 450 nm. PPAR gamma will not crossreact with PPAR delta or PPAR alpha.

Notes:
Peroxisome proliferator-activated receptors (PPARs) are ligand activated nuclear receptors. Three PPAR subtypes have been identified: alpha, delta and gamma. PPARs can be activated by polyunsaturated fatty acids, eicosanoids and various synthtic ligands.

PPAR gamma is primarily expressed in adipose tissue and to a lesser extent in the colon, immune system and the retina. PPAR gamma was first identified as regulator of adipogenesis, but also plays an important role in cellular differentiation, insulin sensitization, atherosclerosis and cancer.

Platform: Microplate reader

Storage instructions: Please refer to protocols.

Components

<table>
<thead>
<tr>
<th>Components</th>
<th>Quantity</th>
</tr>
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<tbody>
<tr>
<td>96-Well Plate Cover</td>
<td>1 unit</td>
</tr>
</tbody>
</table>

96 tests
Function
Receptor that binds peroxisome proliferators such as hypolipidemic drugs and fatty acids. Once activated by a ligand, the receptor binds to a promoter element in the gene for acyl-CoA oxidase and activates its transcription. It therefore controls the peroxisomal beta-oxidation pathway of fatty acids. Key regulator of adipocyte differentiation and glucose homeostasis.

Tissue specificity
Highest expression in adipose tissue. Lower in skeletal muscle, spleen, heart and liver. Also detectable in placenta, lung and ovary.

Involvement in disease
Note=Defects in PPARG can lead to type 2 insulin-resistant diabetes and hypertension. PPARG mutations may be associated with colon cancer. Defects in PPARG may be associated with susceptibility to obesity (OBESITY) [MIM:601665]. It is a condition characterized by an increase of body weight beyond the limitation of skeletal and physical requirements, as the result of excessive accumulation of body fat. Defects in PPARG are the cause of familial partial lipodystrophy type 3 (FPLD3) [MIM:604367]. Familial partial lipodystrophies (FPLD) are a heterogeneous group of genetic disorders characterized by marked loss of subcutaneous (sc) fat from the extremities. Affected individuals show an increased preponderance of insulin resistance, diabetes mellitus and dyslipidemia. Genetic variations in PPARG can be associated with susceptibility to glioma type 1 (GLM1) [MIM:137800]. Gliomas are central nervous system neoplasms derived from glial cells and comprise astrocytomas, glioblastoma multiforme, oligodendrogliomas, and ependymomas. Note=Polymorphic PPARG alleles have been found to be significantly over-represented among a cohort of American patients with sporadic glioblastoma multiforme suggesting a possible contribution to disease susceptibility.

Sequence similarities
Belongs to the nuclear hormone receptor family. NR1 subfamily. Contains 1 nuclear receptor DNA-binding domain.

Cellular localization
Nucleus.

### Components

<table>
<thead>
<tr>
<th>Components</th>
<th>96 tests</th>
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</thead>
<tbody>
<tr>
<td>Polysorbate 20</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor Antibody Binding Buffer (10X)</td>
<td>1 x 3ml</td>
</tr>
<tr>
<td>Transcription Factor Binding Assay Buffer (4X)</td>
<td>1 x 3ml</td>
</tr>
<tr>
<td>Transcription Factor Developing Solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>Transcription Factor Goat Anti-Rabbit HRP Conjugate</td>
<td>1 x 100µl</td>
</tr>
<tr>
<td>Transcription Factor PPAR 96-Well Strip Plate</td>
<td>1 unit</td>
</tr>
<tr>
<td>Transcription Factor PPAR Competitor dsDNA</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor PPAR gamma Positive Control</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor PPAR gamma Primary Antibody</td>
<td>1 vial</td>
</tr>
<tr>
<td>Transcription Factor Reagent A</td>
<td>1 x 120µl</td>
</tr>
<tr>
<td>Transcription Factor Stop Solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 x 5ml</td>
</tr>
</tbody>
</table>
3T3-L1 cells were differentiated to adipocytes with 1μM dexamethasone (ab120743), 1μg x mL⁻¹ insulin (ab123768) and 0.5 mM IBMX (ab120840). From the third day, the cells were grown in normal medium with the addition of only 1μg x mL⁻¹ insulin. From day six, the cells were cultured in normal medium for an additional two days. 40μL of nuclear extracts (ab113474) were tested in duplicates (+/- SD).

Different volumes of positive control with inhibitor (duplicates, +/- SD).

Different volumes of positive control (duplicates, +/-SD).
Panel A: Increasing amounts of positive control (total lysate) are assayed for PPAR gamma DNA-binding activity using ab133101.

Panel B: PPAR gamma DNA-binding assays are performed in the presence of competitive dsDNA. The decrease in signal caused by addition of competitive dsDNA confirms the assay specificity.

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