PPARγ Transcription Factor Assay Kit

Item No. 10006855

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA
GENERAL INFORMATION

Materials Supplied

Kit components may be stored at -20°C prior to use. For long term storage, the Positive Control should be thawed on ice, aliquoted at 25 µl/vial, and stored at -80°C. After opening the kit, we recommend each kit component be stored according to the temperature listed below.

<table>
<thead>
<tr>
<th>Item No.</th>
<th>Item</th>
<th>Quantity/Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10006880</td>
<td>Transcription Factor Binding Assay Buffer (4X)</td>
<td>1 vial/3 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>10007472</td>
<td>Transcription Factor Reagent A</td>
<td>1 vial/120 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>10006881</td>
<td>Transcription Factor PPAR Positive Control</td>
<td>1 vial/150 µl</td>
<td>-80°C</td>
</tr>
<tr>
<td>10006882</td>
<td>Transcription Factor Antibody Binding Buffer (10X)</td>
<td>1 vial/3 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>10006883</td>
<td>Transcription Factor PPAR Primary Antibody</td>
<td>1 vial/120 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>400062</td>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 vial/5 ml</td>
<td>RT</td>
</tr>
<tr>
<td>400035</td>
<td>Polysorbate 20</td>
<td>1 vial/3 ml</td>
<td>RT</td>
</tr>
<tr>
<td>10006885</td>
<td>Transcription Factor PPAR Competitor dsDNA</td>
<td>1 vial/120 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>10006884</td>
<td>Transcription Factor Goat Anti-Rabbit HRP Conjugate</td>
<td>1 vial/120 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>10006887</td>
<td>Transcription Factor PPAR 96-Well Strip Plate</td>
<td>1 plate</td>
<td>4°C</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>1 cover</td>
<td>RT</td>
</tr>
<tr>
<td>10006888</td>
<td>Transcription Factor Developing Solution</td>
<td>1 vial/12 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>10006889</td>
<td>Transcription Factor Stop Solution</td>
<td>1 vial/12 ml</td>
<td>RT</td>
</tr>
</tbody>
</table>

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.
WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

Precautions
Please read these instructions carefully before beginning this assay.
Kit components may be stored at -20°C prior to use. For long term storage, the Positive Control should be thawed on ice, aliquoted at 25 µl/vial and stored at -80°C. If the assay will be used on multiple days, we recommend each kit component be stored according to the temperatures listed in the booklet.

If You Have Problems
Technical Service Contact Information
Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST
In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability
This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied
1. A plate reader capable of measuring absorbance at 450 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of UltraPure water; glass Milli-Q or HPLC-grade water are acceptable
4. 300 mM dithiothreitol (DTT)
5. Nuclear Extraction Kit available from Cayman (Item No. 10009277) or buffers for preparation of nuclear extracts

NOTE: The components in each kit lot have been quality assured and warranted in this specific combination only; please do not mix them with components from other lots.
Background

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors. Three PPAR subtypes have been identified: α, β (also called δ and NUC1) and γ. PPARγ is the most widely studied PPAR and exists in two protein isoforms (γ1 and γ2) due to use of an alternative promoter and alternative splicing.1 PPARγ is primarily expressed in adipose tissue and to a lesser extent in the colon, immune system, and the retina.2 PPARγ was first identified as a regulator of adipogenesis, but also plays an important role in cellular differentiation, insulin sensitization, atherosclerosis, and cancer. Ligands for PPARγ include fatty acids, arachidonic acid metabolites such as 15-deoxy-Δ12,14-PGJ2, as well as thiazolidinediones (TZDs) which include pioglitazone and rosiglitazone.3 TZDs are potent, selective PPARγ agonists that lower the hyperglycemia, hyperinsulinemia and hypertriglyceridemia found in type 2 diabetic subjects.4 The use of these synthetic ligands has increased the understanding of PPARγ’s mechanism of activation and subsequent biological effects. Modulation of PPARγ by TZDs (pioglitazone and rosiglitazone) are presently used in type 2 diabetes as oral antidiabetic drugs.5 By increasing our understanding of PPARγ additional drug candidates may be identified.

About This Assay

Cayman’s PPARγ Transcription Factor Assay 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 (see Figure 1, on page 8). PPARs contained in a nuclear extract bind specifically to the PPRE. PPARγ is detected by addition of specific primary antibody directed against PPARγ. A secondary antibody conjugated to HRP is added to provide a sensitive colorometric readout at 450 nm. The Cayman Chemical PPARγ Transcription Factor Assay detects human, mouse, and rat PPARγ. It will not cross react with PPARδ or PPARα.
Reagent Preparation

1. **Transcription Factor Antibody Binding Buffer (10X)**
   One vial (Item No. 10006882) contains 3 ml of a 10X stock of Transcription Factor Antibody Binding Buffer (ABB) to be used for diluting the primary and secondary antibodies. To prepare a 1X ABB, dilute 1:10 by adding 27 ml of UltraPure water. Store at 4°C for up to six months.

2. **Wash Buffer Concentrate (400X)**
   One vial (Item No. 400062) contains 5 ml of 400X Wash Buffer. Dilute the contents of the vial to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035). **NOTE**: Polysorbate 20 is a viscous liquid and cannot be measured by a pipette. A positive displacement device such as a syringe should be used to deliver small quantities accurately. A smaller volume of Wash Buffer Concentrate can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer). Store at 4°C for up to two months.

3. **Transcription Factor Binding Assay Buffer (4X)**
   One vial (Item No. 10006880) contains 3 ml of a 4X stock of Transcription Factor Binding Assay Buffer (TFB). Prepare Complete TFB Assay Buffer (CTFB) immediately prior to use in 1.5 ml centrifuge tubes or 15 ml conical tubes as outlined in Table 1, on page 10. This buffer is now referred to as CTFB. **It is recommended that the CTFB be used the same day it is prepared.**
Table 1. Preparation of Complete Transcription Factor Binding Assay Buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume/Well</th>
<th>Volume/Strip</th>
<th>Volume/96-well plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>UltraPure water</td>
<td>73 µl</td>
<td>584 µl</td>
<td>7,008 µl</td>
</tr>
<tr>
<td>4X Transcription Factor Binding Assay Buffer</td>
<td>25 µl</td>
<td>200 µl</td>
<td>2,400 µl</td>
</tr>
<tr>
<td>Reagent A (Item No. 10007472)</td>
<td>1 µl</td>
<td>8 µl</td>
<td>96 µl</td>
</tr>
<tr>
<td>300 mM DTT</td>
<td>1 µl</td>
<td>8 µl</td>
<td>96 µl</td>
</tr>
<tr>
<td>Total Required</td>
<td>100 µl</td>
<td>800 µl</td>
<td>9,600 µl</td>
</tr>
</tbody>
</table>

4. Transcription Factor PPARγ Positive Control

One vial (Item No. 10006881) contains 150 µl of clarified cell lysate. This lysate is provided as a positive control for PPARγ activation; it is not intended for plate to plate comparisons. The cell lysate provided is sufficient for 15 reactions and will produce a strong signal (>0.5 AU at 450 nm) when used at 10 µl/well. Serial 2-fold dilutions of this positive control can be used for monitoring the dynamic range of the assay. A decrease in signal may occur with repeated freeze-thaw cycles. It is recommended that the Positive Control be aliquoted at 25-50 µl per vial and stored at -80°C to avoid loss in signal from repeated freeze-thaw cycles.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of PPARγ Positive Control (PC1-PC6) serial dilutions, and unknown samples of nuclear extracts (S1-S40) to be measured in duplicate is given below in Figure 2. We suggest you record the contents of each well on the template sheet provided (see page 22).

Blk - Blank Wells
NSB - Non-specific Binding Wells
PC1-PC6 - Positive Control Wells
S1-S40 - Sample Wells

Figure 2. Sample plate format
**Pipetting Hints**

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

**General Information**

- Plate strips can be used in separated experiments if stored at 4°C properly in the resealable pouch.
- A minimum of two Blk, two NSB, and two PC wells should be included in each assay.
- We recommend using Cayman’s Nuclear Extraction Kit (Item No. 10009277) for preparing your samples.

---

**Performing the Assay**

**Binding of active PPARγ to the consensus sequence**

1. Equilibrate the plate and buffers to room temperature prior to opening. Remove the plate from the foil and select the number of strips needed. The 96-well plate supplied with this kit is ready to use.

   NOTE: If you are not using all of the strips at once, place the unused strips back in the plate packet with the desiccant and store at 4°C.

2. Add appropriate amount of reagents listed below to the designated wells as follows:
   - **Blk** - add 100 μl of CTFB to designated wells.
   - **NSB** - add 100 μl of CTFB to designated wells. Do not add samples or Positive Control to these wells.
   - **Competitor (optional)** - Add 80 μl of CTFB prior to adding 10 μl of Transcription Factor PPAR Competitor dsDNA (Item No. 10006885) to designated wells. Add 10 μl of control cell lysate or sample.
   - **S1-S40** - Add 90 μl of CTFB followed by 10 μl of sample to designated wells.
   - **PC1-PC6** - Add 90 μl of CTFB followed by 10 μl of Positive Control serial dilutions to appropriate wells.

3. Use the cover provided to seal the plate. Incubate overnight at 4°C or one hour at room temperature (incubation for one hour will result in a less sensitive assay).

4. Empty the wells and wash five times with 200 μl of 1X Wash Buffer. After the final wash, tap the plate on a paper towel to remove any residual Wash Buffer.
Addition of Transcription Factor PPARγ Primary Antibody
5. Dilute the Transcription Factor PPARγ Primary Antibody (Item No. 10006883) 1:100 in 1X ABB. Add 100 μl to each well except the Blk wells.
6. Use the adhesive cover sheet provided to seal the plate.
7. Incubate the plate for one hour at room temperature.
8. Empty the wells and wash each well five times with 200 μl of 1X Wash Buffer. After the final wash, tap the plate three to five times on a paper towel to remove any residual Wash Buffer.

Addition of the Transcription Factor Goat Anti-Rabbit HRP Conjugate
9. Dilute the Transcription Factor Goat Anti-Rabbit HRP Conjugate (Item No. 10006884) 1:100 in 1X ABB. Add 100 μl to each well except the Blk wells.
10. Use the adhesive cover provided to seal the plate.
11. Incubate at room temperature.
12. Empty the wells and wash five times with 200 μl of 1X Wash Buffer. After the final wash, tap the plate three to five times on a paper towel to remove any residual Wash Buffer.

Develop and Read the Plate:
13. Add 100 μl of Transcription Factor Developing Solution (Item No. 10006888) to each well.
14. Incubate the plate for 15 to 45 minutes at room temperature with gentle agitation protected from light. Allow the wells to turn medium to dark blue prior to adding Transcription Factor Stop Solution (Item No. 10006889). (This reaction can be monitored by taking absorbance measurements at 655 nm prior to stopping the reactions; An OD_{655} of 0.4-0.5 yields an OD_{450} of approximately 1). NOTE: Do not overdevelop the sample wells; however some PC wells may need to be overdevelop to allow adequate color development in sample wells. In that case, make sure the samples are within the dynamic range of the assay as indicated by at least some of the positive control serial dilutions.
15. Add 100 μl of Stop Solution per well. The solution within the wells will change from blue to yellow after adding the Stop Solution.
16. Read absorbance at 450 nm within five minutes of adding the Stop Solution.
Assay Procedure Summary

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when initially performing the assay.

1. Add reagents to wells as indicated in Table 2.
2. Incubate overnight at 4°C or one hour at room temperature.
3. Wash each well five times with 200 μl of 1X Wash Buffer.
4. Add 100 μl of diluted PPARγ Primary Antibody per well (except Blk wells).
5. Incubate one hour at room temperature.
6. Wash each well five times with 200 μl of 1X Wash Buffer.
7. Add 100 μl of diluted Secondary Antibody (except Blk wells).
8. Incubate one hour at room temperature.
9. Wash each well five times with 200 μl of 1X Wash Buffer.
10. Add 100 μl of Developing Solution per well.
11. Incubate 15 to 45 minutes with gentle agitation.
12. Add 100 μl of Stop Solution per well.
13. Measure the absorbance at 450 nm.

### Table 2. Quick Protocol Guide

<table>
<thead>
<tr>
<th>Steps</th>
<th>Reagent</th>
<th>Blk</th>
<th>NSB</th>
<th>PC1-PC6</th>
<th>Competitor</th>
<th>S1-S40</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Add reagents</td>
<td>CTFB</td>
<td>100 μl</td>
<td>100 μl</td>
<td>90 μl</td>
<td>80 μl</td>
<td>90 μl</td>
</tr>
<tr>
<td></td>
<td>Competitor dsDNA</td>
<td></td>
<td></td>
<td>10 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive Control</td>
<td></td>
<td></td>
<td>10 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Samples</td>
<td></td>
<td></td>
<td>10 μl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Incubate</td>
<td>Cover plate and incubate overnight at 4°C or one hour at RT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Wash</td>
<td>Wash all wells five times</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Add reagents</td>
<td>Primary Antibody</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td></td>
</tr>
<tr>
<td>5. Incubate</td>
<td>Cover plate and incubate one hour at RT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Wash</td>
<td>Wash all wells five times</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Add reagents</td>
<td>Secondary Antibody</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td></td>
</tr>
<tr>
<td>8. Incubate</td>
<td>Cover plate and incubate one hour at RT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Wash</td>
<td>Wash all wells five times</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Add reagents</td>
<td>Developer Solution</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td></td>
</tr>
<tr>
<td>11. Incubate</td>
<td>Monitor development in wells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Add reagents</td>
<td>Stop Solution</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td>100 μl</td>
<td></td>
</tr>
<tr>
<td>13. Read</td>
<td>Read plate at wavelength of 450 nm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**ANALYSIS**

**Performance Characteristics**

**Figure 3. Panel A:** Increasing amounts of positive control (total lysate) are assayed for PPARγ DNA-binding activity using the Cayman's PPARγ Transcription Factor Assay Kit. **Panel B:** PPARγ 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.

**RESOURCES**

**Interferences**

The following reagents were tested for interference in the assay.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Will Interfere (Yes or No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGTA (≤1 mM)</td>
<td>No</td>
</tr>
<tr>
<td>EDTA (≤0.5 mM)</td>
<td>No</td>
</tr>
<tr>
<td>ZnCl (any concentration)</td>
<td>Yes</td>
</tr>
<tr>
<td>DTT (between 1 and 5 mM)</td>
<td>No</td>
</tr>
<tr>
<td>Dimethylsulfoxide (≤1.5%)</td>
<td>No</td>
</tr>
</tbody>
</table>
Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
</table>
| No signal or weak signal in control wells | A. Omission of key reagent  
B. Plate reader settings not correct  
C. Reagent expired  
D. Salt concentrations affected binding between DNA and protein  
E. Developing reagent used cold  
F. Developing reagent not added to correct volume | A. Check that all reagents have been added and in the correct order; perform the assay using the Positive Control  
B. Check wavelength setting on plate reader and change to 450 nm  
C. Check expiration date  
D. Reduce the amount of nuclear extract used in the assay, or reduce the amount of salt in the nuclear extracts (alternatively can perform buffer exchange)  
E. Prewarm the Developing Solution to room temperature prior to use  
F. Check pipettes to ensure correct amount of Developing Solution was added to wells |
| High signal in all wells | A. Incorrect dilution of antibody (too high)  
B. Improper/inadequate washing of wells  
C. Over-developing | A. Check antibody dilutions and use amounts outlined in instructions  
B. Follow the protocol for washing wells using the correct number of times and volumes  
C. Decrease the incubation time when using the developing reagent |
| High background (NSB) | Incorrect dilution of antibody (too high) | Check antibody dilutions and use amounts outlined in the instructions |

References

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman’s Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©04/21/2017, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.