PPARα, δ, γ Complete Transcription Factor Assay Kit

Item No. 10008878

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Customer Service 800.364.9897
Technical Support 888.526.5351
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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
10006880	Transcription Factor Binding Assay Buffer (4X)	1 vial/3 ml	4°C
10007472	Transcription Factor Reagent A	1 vial/120 µl	-20°C
10007441	Transcription Factor PPARα Positive Control	1 vial/150 µl	-80°C
10007443	Transcription Factor PPARδ Positive Control	1 vial/150 µl	-80°C
10006881	Transcription Factor PPARγ Positive Control	1 vial/150 µl	-80°C
10006882	Transcription Factor Antibody Binding Buffer (10X)	1 vial/3 ml	4°C
10007442	Transcription Factor PPARα Primary Antibody	1 vial/120 µl	-20°C
10007444	Transcription Factor PPARδ Primary Antibody	1 vial/120 µl	-20°C
10006883	Transcription Factor PPARγ Primary Antibody	1 vial/120 µl	-20°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
10006885	Transcription Factor PPAR Competitor dsDNA	1 vial/120 µl	-20°C
10006884	Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 vial/120 µl	-20°C
10006887	Transcription Factor PPAR 96-Well Strip Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 cover	RT
10006888	Transcription Factor Developing Solution	1 vial/12 ml	4°C
10006889	Transcription Factor Stop Solution	1 vial/12 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. 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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's PPARα, δ, γ Complete Transcription Factor Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (e.g. safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

E-Mail: techserv@caymanchem.com

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 in the **Materials Supplied** section (see page 3) 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. An orbital microplate shaker
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. 300 mM dithiothreitol (DTT)

INTRODUCTION

Background

Peroxisome Proliferator-activated Receptors (PPARs) are ligand-activated transcription factors belonging to the large superfamily of nuclear receptors.[1,2] They are activated by a variety of fatty acids and fatty acid derivatives such as prostaglandins and leukotrienes. PPARs play pivotal roles in the regulation of lipid metabolism and homeostasis and are important indirect as well as direct regulators of cellular insulin sensitivity.[3] There are three major PPAR isoforms; PPARα, PPARγ, and PPARδ/β which all bind to PPAR responsive elements (PPRE's) as heterodimers with retinoid X receptor (RXR), another member of the nuclear receptor superfamily. PPARα primarily activates genes encoding proteins involved in fatty acid oxidation, while PPARγ primarily activates genes directly involved in lipogenic pathway and insulin signaling.[1,4,5] Members of the PPAR family are important direct targets of many antidiabetic and hypolipidemic drugs.[6]

About This Assay

Cayman's PPARα, δ, γ Complete Transcription Factor Assay Kit is a sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A specific double stranded DNA (dsDNA) sequence containing the PPAR response element is immobilized onto the wells of a 96-well plate (see Figure 1, on page 7). PPARs contained in a sample bind specifically to the immobilized PPAR response element and are detected by addition of specific primary antibodies directed against the individual PPAR isoforms. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. Cayman's PPARα, δ, γ Complete Transcription Factor Assay Kit is designed to investigate all three PPAR isoforms in 12 samples on a single plate.

Cayman offers a Nuclear Extraction Kit (Item No. 10009277) to prepare nuclear extracts for use in this kit.

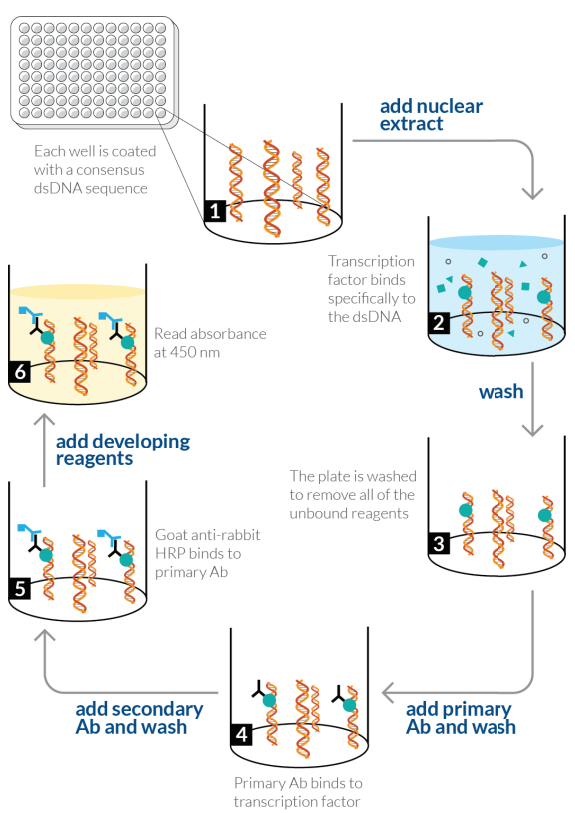


Figure 1. Schematic of the Transcription Factor Binding Assay

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Transcription Factor Antibody Binding Buffer (10X)**

   This vial (Item No. 10006882) contains 3 ml of 10X Transcription Factor Antibody Binding Buffer (ABB) to be used for diluting the primary and secondary antibodies. To prepare 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)**

   This 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). Store at 4°C for up to two months. *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.*

3. **Complete Transcription Factor Binding Assay Buffer**

   Prepare 10 ml of Complete Transcription Factor Binding Assay Buffer (CTFB) by adding 2.5 ml of Transcription Factor Binding Buffer (4X) (Item No. 10006880), 0.1 ml of Transcription Factor Reagent A (Item No. 10007472), and 0.1 ml of 300 mM DTT to 7.3 ml of ultrapure water. Scale as necessary. *It is recommended that the CTFB be used the same day it is prepared.*

4. **Transcription Factor PPARα, δ, γ Positive Controls**

   Each vial (Item Nos. 10007441, 10007443, and 10006881) contains an isoform specific positive control. These positive controls will provide a signal >0.5 AU at 450 nm when used at 10 µl/well. It is recommended that the positive controls be aliquoted at 50 µl per vial and stored at -80°C to avoid loss of signal from repeated freeze/thaw cycles.

5. **Transcription Factor PPARα, δ, γ Primary Antibodies**

   Each vial (Item Nos. 10007442, 10007444, and 10006883) contains an isoform specific primary antibody. Dilute each primary antibody 1:100 in 1X ABB. For a full plate containing all three isoforms as shown in Figure 2 (see page 10), dilute 50 µl of each primary antibody with 4.95 ml of 1X ABB. Adjust volumes as required by experimental setup and prepare diluted antibodies immediately before use. Discard any unused diluted antibody.

6. **Transcription Factor Goat Anti-Rabbit HRP Conjugate**

   This vial (Item No. 10006884) contains an HRP conjugated secondary antibody. Dilute 1:100 in 1X ABB. For a full plate, dilute the 120 µl with 11.88 ml of 1X ABB. Adjust volumes as required by experimental setup and prepare diluted conjugate immediately before use. Discard any unused diluted conjugate.

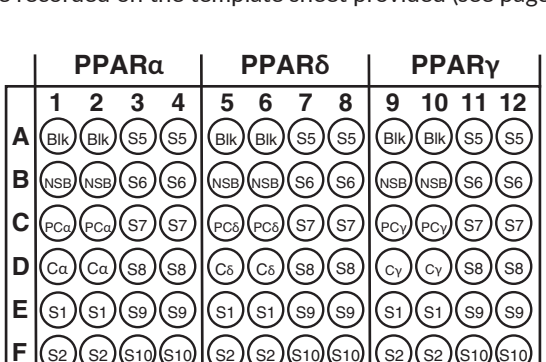
7. **Transcription Factor PPAR Competitor dsDNA (optional)**

   This vial (Item No. 10006885) contains the PPAR consensus sequence. This optional competitor dsDNA will provide a reduction in signal of >25% when used at 10 µl/well and added prior to the positive control or sample. The competitor dsDNA is ready to use as supplied.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout of PPAR Positive Control (PC), Competitor dsDNA (C1), and samples (S1-S12) to be measured in duplicate is given below in Figure 2. It is suggested that the contents of each well be recorded on the template sheet provided (see page 18).



Blk = Blank Wells
NSB = Non-specific Binding Wells
PC = Positive Control Wells
C = Competitor dsDNA Wells
S1-S12 = 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

- For each plate or set of strips it is recommended that two Blk, two isoform specific NSB, and two isoform specific PC wells be included.
- If you are not using all of the strips at once, place the unused strips back in the plate packed and store at 4°C. Be sure the packet is sealed with the desiccant inside.

Performing the Assay

Addition of Positive Controls and Samples

1. Equilibrate the plate and buffers to room temperature.
2. Prepare the CTFB as outlined on page 8.
3. Add appropriate amount of reagent(s) listed below to the designated wells as follows:

Reagent	Blk	NSB	C	PC	S1-S12
CTFB	100 µl	100 µl	80 µl	90 µl	90 µl
Competitor dsDNA*	--	--	10 µl	--	--
Positive Controls	--	--	10 µl	10 µl	--
Samples	--	--	--	--	10 µl

**NOTE: Optional competitor dsDNA must be added prior to adding the positive controls or samples*

4. Use the 96-Well Cover Sheet (Item No. 400012) provided to seal the plate. Incubate overnight at 4°C or one hour at room temperature on an orbital shaker.
5. Empty the wells and rinse five times with 200 µl of 1X wash buffer. After the final wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.

Addition of the Primary Antibodies

1. Add 100 µl of diluted primary antibody to all wells for each isoform except the Blk wells.
2. Seal the plate with cover sheet and incubate the plate for one hour at room temperature on an orbital shaker.
3. Empty the wells and rinse five times with 200 µl of 1X wash buffer. After the final wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.

Addition of the HRP Conjugate

1. Add 100 µl of diluted HRP conjugate to all wells for except the Blk wells.
2. Seal the plate with cover sheet and incubate the plate for one hour at room temperature on an orbital shaker.

Develop and Read the Plate:

1. Empty the wells and rinse five times with 200 µl of 1X wash buffer. After the final wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of Transcription Factor Developing Solution (Item No. 10006888) each well of the plate.
3. Seal the plate with cover sheet and incubate the plate for 30 minutes at room temperature on an orbital shaker protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of Transcription Factor Stop Solution (Item No. 10006889) to each well of the plate. Blue Wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*
5. Measure absorbance at 450 nm within five minutes of adding the stop solution. Blank the plate reader according to the manufacturer's requirements using the blank wells.

Assay Procedure Summary

NOTE: Follow the detailed procedure when initially performing the assay.

1. Add 90 µl CTFB per sample well (80 µl if adding competitor dsDNA) and 100 µl to Blk and NSB wells.
2. Add 10 µl of competitor dsDNA (optional) to appropriate wells.
3. Add 10 µl of positive control or sample to appropriate wells.
4. Incubate overnight at 4°C or one hour at room temperature on an orbital shaker.
5. Wash five times with 200 µl of 1X Wash Buffer.
6. Add 100 µl of diluted primary antibody per well (except Blk wells).
7. Incubate one hour at room temperature on an orbital shaker.
8. Wash five times with 200 µl of 1X Wash Buffer.
9. Add 100 µl of diluted HRP conjugate per well (except Blk wells).
10. Incubate one hour at room temperature on an orbital shaker.
11. Wash five times with 200 µl of 1X Wash Buffer.
12. Add 100 µl of developing solution per well.
13. Incubate 30 minutes on an orbital shaker.
14. Add 100 µl of stop solution per well.
15. Measure the absorbance at 450 nm.

ANALYSIS

Calculations

1. Subtract the absorbance of the NSB wells from all other wells for each isoform except Blk wells. These are the corrected absorbance values.
2. Calculate percent inhibition of the competitor dsDNA (optional):

$$\% \text{ inhibition} = \left[1 - \frac{\text{corrected absorbance with competitor dsDNA}}{\text{corrected absorbance without competitor dsDNA}}\right] \times 100$$

Performance Characteristics

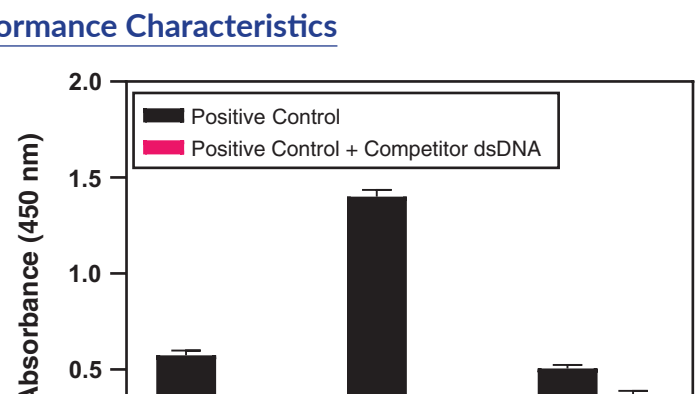


Figure 3. Inhibition with Competitor dsDNA

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

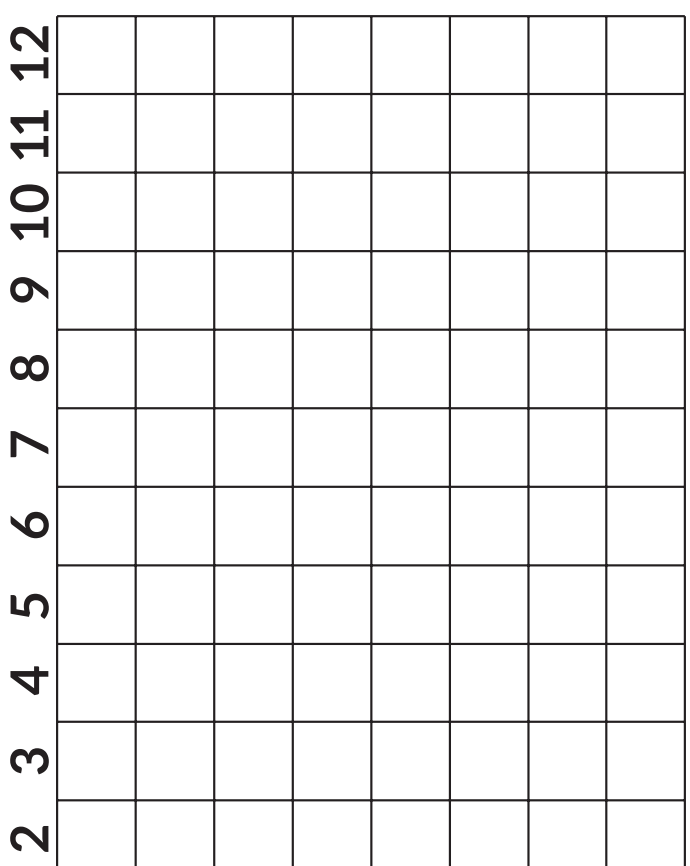
Reagent	Will Interfere (Yes or No)
EGTA (≤1 mM)	No
EDTA (≤0.5 mM)	No
ZnCl (any concentration)	Yes
DTT (between 1 and 5 mM)	No
DMSO (≤1.5%)	No

Troubleshooting

Problem	Possible Causes
No signal or weak signal in all wells	A. Omission or incorrect dilution of key reagent B. Developing reagent used cold
High signal in all wells	A. Incorrect dilution of antibody B. Improper/inadequate washing C. Plate developed for too long
Weak signal in sample wells	A. Sample concentration is too low B. Salt concentrations affecting binding between DNA and protein

References

1. Desvergne, B. and Wahli, W. Peroxisome proliferator-activated receptors: Nuclear control of metabolism. *Endocr. Rev.* **20**, 649-688 (1999).
2. Clark, R.B. The role of PPARs in inflammation and immunity. *J. Leukoc. Biol.* **71**, 388-400 (2002).
3. Olefsky, J.M. Nuclear receptor minireview series. *J. Biol. Chem.* **276(40)**, 36863-36864 (2001).
4. Duplus, E., Glorian, M., and Forest, C. Fatty acid regulation of gene transcription. *J. Biol. Chem.* **275(40)**, 30749-30752 (2000).
5. Gervois, P., Torra, I.P., Fruchart, J.-C., *et al.* Regulation of lipid and lipoprotein metabolism by PPAR activators. *Clin. Chem. Lab. Med.* **38(1)**, 3-11 (2000).
6. Brown, P.J., Smith-Oliver, T.A., Charifson, P.S., *et al.* Identification of peroxisome proliferator-activated receptor ligands from a biased chemical library. *Chemistry & Biology* **4**, 909-918 (1997).



NOTES

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.