

**PPAR $\alpha$ ,  $\delta$ ,  $\gamma$  Complete  
Transcription Factor Assay Kit**

Catalog No. 10008878

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## GENERAL INFORMATION

### Materials Supplied

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

Catalog Number	Item	Quantity	Storage
10006880	Transcription Factor Binding Assay Buffer (4X)	1 vial	4°C
10007472	Transcription Factor Reagent A	1 vial	-20°C
10008892	Transcription Factor Complete PPAR $\alpha$ Positive Control	1 vial	-80°C
10008893	Transcription Factor Complete PPAR $\delta$ Positive Control	1 vial	-80°C
10008894	Transcription Factor Complete PPAR $\gamma$ Positive Control	1 vial	-80°C
10006882	Transcription Factor Antibody Binding Buffer (10X)	1 vial	4°C
10008895	Transcription Factor Complete PPAR $\alpha$ Primary Antibody	1 vial	-20°C
10008896	Transcription Factor Complete PPAR $\delta$ Primary Antibody	1 vial	-20°C
10008897	Transcription Factor Complete PPAR $\gamma$ Primary Antibody	1 vial	-20°C
400062	Wash Buffer Concentrate (400X)	1 vial	4°C
400035	Tween 20	1 vial	RT
10006885	Transcription Factor PPAR Specific Competitor dsDNA	1 vial	-20°C
10006884	Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 vial	-20°C
10006887	Transcription Factor PPAR 96-Well Plate	1 plate	4°C
400012	96-Well Cover Sheet	1 cover	RT
10006888	Transcription Factor Developing Solution	1 vial	4°C
10006889	Transcription Factor Stop Solution	1 vial	4°C

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 laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

## 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 Controls should be thawed on ice, aliquoted at 25 µl/vial and stored at -80°C. After use we recommend each kit component be stored according to the temperatures listed in the booklet.

**For research use only. Not for human or diagnostic use.**

## If You Have Problems

### Technical Service Contact Information

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

**Fax:** 734-971-3641

**E-Mail:** 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 repeat 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 (Catalog No. 10009277) or buffers for preparation of nuclear extracts (see page 8-10)

*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 transcription factors belonging to the large superfamily of nuclear receptors.<sup>1,2</sup> 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.<sup>3</sup> There are three major PPAR isotypes; PPAR $\alpha$ , PPAR $\gamma$ , and PPAR $\delta/\beta$  which all bind to PPAR responsive elements (PPRE's) as heterodimers with RXR, another member of the nuclear receptor superfamily. PPAR $\alpha$  primarily activates genes encoding proteins involved in fatty acid oxidation, while PPAR $\gamma$  primarily activates genes directly involved in lipogenic pathway and insulin signaling.<sup>1,4,5</sup> Members of the PPAR family are important direct targets of many antidiabetic and hypolipidemic drugs.<sup>6</sup>

## About This Assay

Cayman's PPAR $\alpha$ ,  $\delta$ ,  $\gamma$  Complete Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. 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 PPAR response element is immobilized onto the bottom of wells of a 96-well plate (see Figure 1, on page 7). PPARs contained in a nuclear extract, bind specifically to the PPAR response element. PPAR $\alpha$ ,  $\delta$ , or  $\gamma$  are detected by addition of specific primary antibodies directed against the individual PPARs. A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. Cayman's PPAR $\alpha$ ,  $\delta$ ,  $\gamma$  Complete Transcription Factor Assay detects PPAR $\alpha$ ,  $\delta$ , and  $\gamma$ .

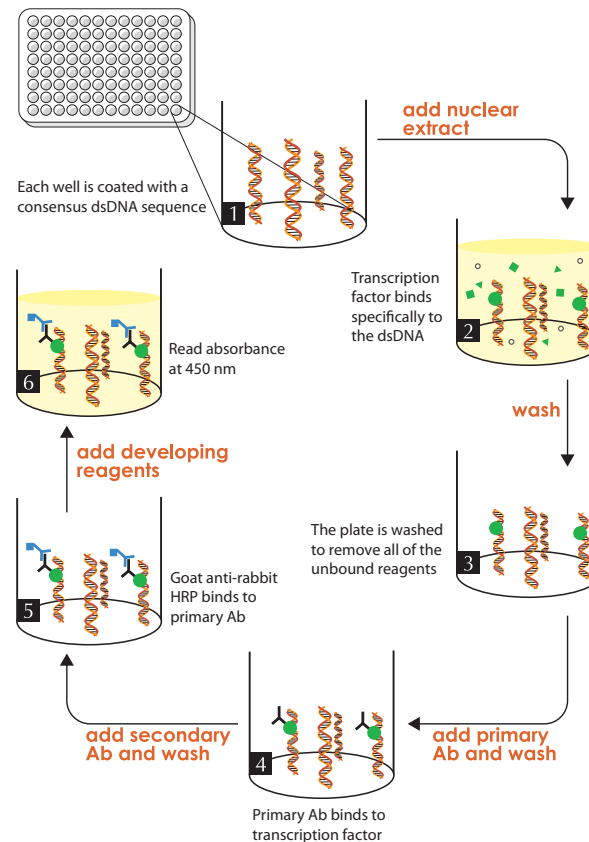


Figure 1. Schematic of the Transcription Factor Binding Assay

## PRE-ASSAY PREPARATION

### Sample Buffer Preparation

All buffers and reagents below required for preparation of Nuclear Extracts can be purchased directly from Cayman. Catalog numbers for each item are in the Related Products section on page 25. Alternatively, Cayman's Nuclear Extraction Kit (Catalog No. 10009277) can be used to isolate Nuclear Proteins.

#### 1. PBS (10X)

Dissolve 80 g NaCl, 2.0 g KCl, 14.4 g  $\text{Na}_2\text{HPO}_4$ , and 2.4 g  $\text{KH}_2\text{PO}_4$  in 800 ml distilled  $\text{H}_2\text{O}$ .

Adjust pH to 7.4 with HCl

Adjust volume to 1 L with  $\text{H}_2\text{O}$

#### 2. PBS (1X)

Dilute 100 ml of 10X stock with 900 ml distilled  $\text{H}_2\text{O}$

#### 3. Nuclear Extraction Phosphatase Inhibitor Cocktail (50X)

1 M NaF, 0.05 M  $\beta$ -glycerophosphate, and 0.05 M  $\text{Na}_3\text{VO}_4$

Store at  $-80^\circ\text{C}$

#### 4. PBS/Phosphatase Inhibitor Solution

Add 200  $\mu\text{l}$  of 50X Phosphatase Inhibitor Solution to 10 ml of 1X PBS, mix well, and keep on ice. Make fresh daily.

#### 5. Nuclear Extraction Protease Inhibitor Cocktail (100X)

10 mM AEBSF

0.5 mM Bestatin

0.2 mM Leupeptin Hemisulfate Salt

0.15 mM E-64

0.1 mM Pepstatin A

0.008 mM Aprotinin from Bovine Lung

Made in DMSO, store at  $-80^\circ\text{C}$

#### 6. Nuclear Extraction Hypotonic Buffer (10X)

100 mM HEPES, pH 7.5, containing 40 mM NaF, 100  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , and 1 mM EDTA

Store at  $4^\circ\text{C}$

#### 7. Complete Extraction Hypotonic Buffer (1X)

Prepare as outlined in Table 1. The phosphatase and protease inhibitors lose activity shortly after dilution; therefore any unused 1X Complete Extraction Hypotonic Buffer should be discarded.

Reagent	150 mm plate $\sim 1.5 \times 10^7$ cells
Hypotonic Buffer (10X)	100 $\mu\text{l}$
Phosphatase Inhibitors (50X)	20 $\mu\text{l}$
Protease Inhibitors (100X)	10 $\mu\text{l}$
Distilled Water	870 $\mu\text{l}$
Total Volume	1,000 $\mu\text{l}$

Table 1. Preparation of Complete Extraction Hypotonic Buffer

## 8. Nuclear Extraction Nonidet P-40 (10%)

Nonidet P-40 or suitable substitute at a concentration of 10% (v/v) in H<sub>2</sub>O

Store at room temperature

## 9. Nuclear Extraction Buffer (2X)

20 mM HEPES, pH 7.9, containing, 0.2 mM EDTA, 3 mM MgCl<sub>2</sub>, 840 mM NaCl, and 20% glycerol (v/v)

Store at 4°C

## 10. Complete Nuclear Extraction Buffer (1X)

Prepare as outlined in Table 2. Some of the phosphatase and protease inhibitors lose activity shortly after dilution; therefore any remaining 1X Extraction Buffer should be discarded.

Reagent	150 mm plate ~1.5 x 10 <sup>7</sup> cells
Nuclear Extraction Buffer (2X)	50 µl
Protease Inhibitors (100X)	1.0 µl
Phosphatase Inhibitors (50X)	2.0 µl
DTT (10 mM)	5 µl
Distilled Water	42 µl
Total Volume	100 µl

**Table 2. Preparation of Complete Nuclear Extraction Buffer**

## Purification of Cellular Nuclear Extracts

Cayman's Nuclear Extraction Kit (Catalog No. 10009277) can be used to isolate nuclear proteins. Alternatively, the procedure described below can be used for a 15 ml cell suspension grown in a T75 flask or adherent cells (100 mm dish 80-90% confluent) where 10<sup>7</sup> cells yields approximately 50 µg of nuclear protein.

1. Collect ~10<sup>7</sup> cells in pre-chilled 15 ml tubes.
2. Centrifuge suspended cells at 300 x g for five minutes at 4°C.
3. Discard the supernatant. Resuspend cell pellet in 5 ml of ice-cold PBS/Phosphatase Inhibitor Solution and centrifuge at 300 x g for five minutes at 4°C. Repeat one time.
4. Discard the supernatant. Add 500 µl ice-cold 1X Hypotonic buffer. Mix gently by pipetting and transfer resuspended pellet to a pre-chilled 1.5 ml microcentrifuge tube.
5. Incubate cells on ice for 15 minutes allowing cells to swell.
6. Add 50 µl of 10% Nonidet P-40 (or suitable substitute). Mix gently by pipetting.
7. Centrifuge for 30 seconds (pulse spin) at 4°C in a microcentrifuge. Transfer the supernatant which contains the cytosolic fraction to a new tube and store at -80°C.
8. Resuspend the pellet in 50 µl ice-cold Complete Nuclear Extraction Buffer (1X) (with protease and phosphatase inhibitors). Vortex 15 seconds at highest setting then gently rock the tube on ice for 15 minutes using a shaking platform. Vortex sample for 30 seconds at highest setting and gently rock for an additional 15 minutes.
9. Centrifuge at 14,000 x g for 10 minutes at 4°C. The supernatant contains the nuclear fraction. Aliquot to clean chilled tubes, flash freeze and store at -80°C. Avoid freeze/thaw cycles. The extracts are ready to use in the assay.
10. Keep a small aliquot of the nuclear extract to quantitate the protein concentration.

## Reagent Preparation

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

One vial (Catalog 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 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 (Catalog 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 Tween 20 (Catalog No. 400035). *NOTE: Tween 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 Tween 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 (Catalog No. 10006880) contains 3 ml of a 4X stock of Transcription Factor Binding Assay Buffer (TFB). Prepare CTFB immediately prior to use in 1.5 ml centrifuge tubes or 15 ml conical tubes as outlined in Table 3, on page 13. This buffer is now referred to as Complete Transcription Factor Binding Assay Buffer (CTFB). *It is recommended that the CTFB be used the same day it is prepared.*

Component	Volume/Well	Volume/Strip	Volume/ 96-well plate
UltraPure Water	73 $\mu$ l	584 $\mu$ l	7,008 $\mu$ l
4X Transcription Factor Binding Assay Buffer	25 $\mu$ l	200 $\mu$ l	2,400 $\mu$ l
Reagent A (Catalog No. 10007472)	1 $\mu$ l	8 $\mu$ l	96 $\mu$ l
300 mM DTT	1 $\mu$ l	8 $\mu$ l	96 $\mu$ l
Total Required	100 $\mu$ l	800 $\mu$ l	9,600 $\mu$ l

**Table 3. Preparation of Complete Transcription Factor Binding Assay Buffer**

### 4. PPAR $\alpha$ , $\delta$ , $\gamma$ Positive Controls

One vial (Catalog Nos. 10008892, 10008893, and 10008894) contains 50  $\mu$ l of clarified cell lysate. These lysates serve as a positive control for PPAR $\alpha$ ,  $\delta$ , and  $\gamma$ , respectively; it is not intended for plate to plate comparisons. The Positive Control provided is sufficient for 5 reactions and will provide a strong signal (>0.5 AU at 450 nm) when used at 10  $\mu$ l/well. When using this Positive Control, a decrease in signal may occur with repeated freeze/thaw cycles. It is recommended that the Positive Control be aliquoted at 25  $\mu$ l per vial and stored at -80°C to avoid loss in signal from repeated freeze/thaw cycles.

### 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 of nuclear extracts (S1 through S44) 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 27).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44
E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37	NSB	NSB
F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38	PC	PC
G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39	Blk	Blk
H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40	C1	C1

S1-S44 - Sample Wells

NSB - Non-specific Binding Wells

PC - Positive Control Wells

Blk - Blank Wells

C1 - Competitor dsDNA Wells

Figure 2. Sample plate format

### Pipetting Hints

- It is recommended that an adjustable pipette be used to deliver reagents to the wells.
- 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

- It is not necessary to use all the wells on the plate at one time; however a Positive Control should be run every time.
- For each plate or set of strips it is recommended that two Blanks (Blk), two Non-Specific Binding (NSB), and two Positive Control wells be included.

### Performing the Assay

#### Binding of active PPAR $\alpha$ , $\delta$ , and/or $\gamma$ 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 and store at 2-4°C. Be sure that the packet is sealed with the desiccant inside.*

2. Prepare the Complete Transcription Factor Buffer (CTFB) as outlined in Table 3, on page 13.

- Add appropriate amount of reagent(s) listed below to the designated wells as follows:

**Blank Wells (Blk)** - add 100 µl of CTFB to designated wells.

**Non-specific Binding Wells (NSB)** - add 100 µl of CTFB to designated wells. Do not add samples or Positive Control to these wells.

**Competitor Wells (C1)** - Add 80 µl of CTFB prior to adding 10 µl of Competitor dsDNA (Catalog No. 10006885) to designated wells. Add 10 µl of control cell lysate, or unknown sample.

*NOTE: Competitor dsDNA must be added prior to adding the positive control or nuclear extracts.*

**Sample Wells (S1-S44)** - Add 90 µl of CTFB followed by 10 µl of Nuclear Extract to designated wells. A protocol for isolation of nuclear extracts is given on page 11.

**Positive Control Wells (PC)** - Add 90 µl of CTFB followed by 10 µl of Positive Control to appropriate wells.

- Use the cover provided to seal the plate. Incubate overnight at 4°C or one hour at room temperature without agitation (incubation for one hour will result in a less sensitive assay).
- Empty the wells and wash five times with 200 µl of 1X Wash Buffer. After each wash empty the wells in the sink.

After the final wash (wash #5), tap the plate on a paper towel to remove any residual Wash Buffer.

### Addition of PPAR Primary Antibody

- Dilute the PPAR Primary Antibody (Catalog Nos. 10008895, 10008896, and 10008897) 1:100 in 1X Antibody Binding Buffer (ABB) as outlined in Table 4, on page 17. Add 100 µl of diluted PPAR Primary Antibodies to each well except the Blank (Blk) wells.

Component	Volume/Well	Volume/Strip	Volume/96-well plate
1X ABB	99 µl	792 µl	9,504 µl
PPAR Primary Antibody	1 µl	8 µl	96 µl
Total required	100 µl	800 µl	9,600 µl

**Table 4. Dilution of Primary Antibody**

- Use the adhesive cover provided to seal the plate.
- Incubate the plate for one hour at room temperature without agitation.
- Empty the wells and wash each well five times with 200 µl of 1X Wash Buffer. After each wash, empty the contents of the plate into the sink. After the final wash (wash #5), tap the plate three to five times on a paper towel to remove any residual Wash Buffer.

### Addition of the Goat Anti-Rabbit HRP Conjugate

- Dilute the HRP-Conjugated Secondary Antibody (Catalog No. 10006884) 1:100 in 1X Antibody Binding Buffer (ABB) as outlined in Table 5 below. Add 100 µl of diluted secondary antibody to each well except the Blank (Blk) wells.

Component	Volume/Well	Volume/Strip	Volume/96-well plate
1X ABB	99 µl	792 µl	9,504 µl
Goat Anti-Rabbit HRP Conjugate	1 µl	8 µl	96 µl
Total required	100 µl	800 µl	9,600 µl

**Table 5. Dilution of Secondary Antibody**

- Use the adhesive cover provided to seal the plate.
- Incubate for one hour at room temperature without agitation.
- Empty the wells and wash five times with 200 µl of 1X Wash Buffer. After each wash, empty the contents of the plate into the sink. After the final wash (wash #5), tap the plate three to five times on a paper towel to remove any residual Wash Buffer.

## Develop and Read the Plate:

1. To each well being used add 100  $\mu$ l of Developing Solution (Catalog No. 10006888), which has been equilibrated to room temperature.
2. 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 Stop Solution (Catalog No. 10006889). (This reaction can be monitored by taking absorbance measurements at 655 nm prior to stopping the reactions; An OD<sub>655</sub> of 0.4 to 0.5 yields an OD<sub>450</sub> of approximately 1). Monitor development of sample wells to ensure adequate color development prior to stopping the reaction. *NOTE: Do not overdevelop; however Positive Control wells may need to overdevelop to allow adequate color development in sample wells.*
3. Add 100  $\mu$ l of Stop Solution per well being used. The solution within the wells will change from blue to yellow after adding the Stop Solution.
4. Read 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: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when initially performing the assay.*

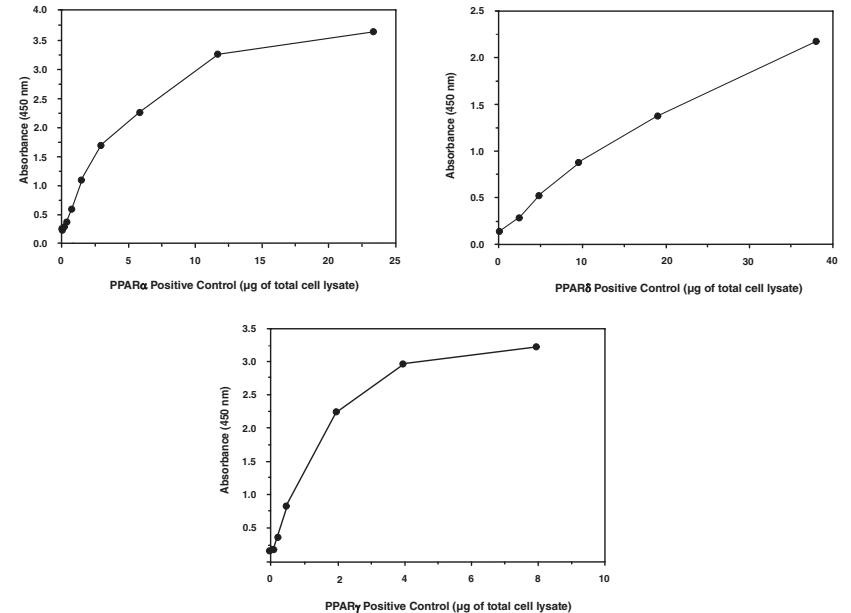
1. Prepare Complete Transcription Factor Binding Buffer (CTFB) as described in the Pre-Assay Preparation section, Table 3 on page 13.
2. Add 90  $\mu$ l CTFB per sample well (80  $\mu$ l if adding Competitor dsDNA), 100  $\mu$ l to Blank (Blk) and non-specific binding (NSB) wells).
3. Add 10  $\mu$ l of Competitor dsDNA (optional) to appropriate wells.
4. Add 10  $\mu$ l of Positive Control to appropriate wells.
5. Add 10  $\mu$ l of Sample containing PPAR $\alpha$ ,  $\delta$ , and/or  $\gamma$  to appropriate wells.
6. Incubate overnight at 4°C without agitation.
7. Wash each well five times with 200  $\mu$ l of 1X Wash Buffer.
8. Add 100  $\mu$ l of diluted PPAR $\alpha$ ,  $\delta$ , and/or  $\gamma$  Primary Antibody per well (except Blank wells).
9. Incubate one hour at room temperature without agitation.
10. Wash each well five times with 200  $\mu$ l of 1X Wash Buffer.
11. Add 100  $\mu$ l of diluted Goat Anti-Rabbit HRP Conjugate (except Blank wells).
12. Incubate one hour at room temperature without agitation.
13. Wash each well five times with 200  $\mu$ l of 1X Wash Buffer.
14. Add 100  $\mu$ l of Developing Solution per well.
15. Incubate 15 to 45 minutes with gentle agitation.
16. Add 100  $\mu$ l of Stop Solution per well.
17. Measure the absorbance at 450 nm.

Steps	Reagent	Blk	NSB	PC	C1	S1-S44
1. Add reagents	CTFB	100 $\mu$ l	100 $\mu$ l	90 $\mu$ l	80 $\mu$ l	90 $\mu$ l
	Competitor dsDNA				10 $\mu$ l	
	Positive Control			10 $\mu$ l	10 $\mu$ l	
	Samples					10 $\mu$ l
2. Incubate	Cover plate and incubate overnight at 4°C without agitation					
3. Wash	Wash all wells five times					
4. Add reagents	Primary Antibody		100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
5. Incubate	Cover plate and incubate one hour at room temperature without agitation					
6. Wash	Wash all wells five times					
7. Add reagents	Goat Anti-Rabbit HRP Conjugate		100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
8. Incubate	Cover plate and incubate one hour at room temperature without agitation					
9. Wash	Wash all wells five times					
10. Add reagents	Developer Solution	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
11. Incubate	Monitor development in wells					
12. Add reagents	Stop Solution	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l	100 $\mu$ l
13. Read	Read plate at wavelength of 450 nm					

**Table 6. Quick Protocol Guide**

## ANALYSIS

### Performance Characteristics



**Figure 3. Schematic of the Transcription Factor Binding Assay**

Increasing amounts of positive control (total lysate) are assayed for PPAR $\alpha$ ,  $\delta$ , and  $\gamma$  DNA-binding activity using the Cayman's PPAR $\alpha$ ,  $\delta$ ,  $\gamma$  Complete Transcription Factor Assay Kit.

### Interferences

The following reagents were tested for interference in the assay.

Reagent	Will Interfere (Yes or No)
EGTA ( $\leq 1$ mM)	No
EDTA ( $\leq 0.5$ mM)	No
ZnCl (any concentration)	Yes
DTT (between 1 and 5 mM)	No
Dimethylsulfoxide ( $\leq 1.5\%$ )	No

### Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal or weak signal in all wells	<ul style="list-style-type: none"> <li>A. Omission of key reagent</li> <li>B. Plate reader settings not correct</li> <li>C. Reagent/reagents expired</li> <li>D. Salt concentrations affected binding between DNA and protein</li> <li>E. Developing reagent used cold</li> <li>F. Developing reagent not added to correct volume</li> </ul>	<ul style="list-style-type: none"> <li>A. Check that all reagents have been added and in the correct order. Perform the assay using the positive control</li> <li>B. Check wavelength setting on plate reader and change to 450 nm</li> <li>C. Check expiration date on reagents</li> <li>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)</li> <li>E. Prewarm the Developing Solution to room temperature prior to use</li> <li>F. Check pipettes to ensure correct amount of developing solution was added to wells</li> </ul>
High signal in all wells	<ul style="list-style-type: none"> <li>A. Incorrect dilution of antibody (too high)</li> <li>B. Improper/inadequate washing of wells</li> <li>C. Over-developing</li> </ul>	<ul style="list-style-type: none"> <li>A. Check antibody dilutions and use amounts outlined in instructions</li> <li>B. Follow the protocol for washing wells using the correct number of times and volumes</li> <li>C. Decrease the incubation time when using the developing reagent</li> </ul>
High background (NSB)	Incorrect dilution of antibody (too high)	Check antibody dilutions and use amounts outlined in the instructions

Problem cont.	Possible Causes cont.	Recommended Solutions cont.
Weak signal in sample wells	<p>A. Sample concentration is too low</p> <p>B. Incorrect dilution of antibody</p> <p>C. Salt concentrations affecting binding between DNA and protein</p>	<p>A. Increase the amount of nuclear extract used. Loss of signal can occur with multiple freeze/thaw cycles of the sample. Prepare fresh nuclear extracts and aliquot as outlined in product insert</p> <p>B. Check antibody dilutions and use amounts outlined in the instructions</p> <p>C. 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)</p>

## References

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## Related Products

ATF2 (Phospho-Thr<sup>69/71</sup>) Transcription Factor Assay Kit - Cat. No. 600130  
 CREB Transcription Factor Assay Kit - Cat. No. 10006909  
 CREB (Phospho-Ser<sup>133</sup>) Transcription Factor Assay Kit - Cat. No. 10009846  
 HIF-1 $\alpha$  Transcription Factor Assay Kit - Cat. No. 10006910  
 Liver X Receptor  $\beta$  Transcription Factor Assay Kit - Cat. No. 10011119  
 NF- $\kappa$ B (human p50) Transcription Factor Assay Kit - Cat. No. 10006912  
 NF- $\kappa$ B (human p50/p65) Combo Transcription Factor Assay Kit - Cat. No. 10011223  
 NF- $\kappa$ B (p65) Transcription Factor Assay Kit - Cat. No. 10007889  
 Nuclear Extraction Buffer (2X) - Cat. No. 10009306  
 Nuclear Extraction Hypotonic Buffer (10X) - Cat. No. 10009301  
 Nuclear Extraction Kit - Cat. No. 10009277  
 Nuclear Extraction Nonidet P-40 (10%) - Cat. No. 10009307  
 Nuclear Extraction PBS (10X) - Cat. No. 10009304  
 Nuclear Extraction Phosphatase Inhibitors (50X) - Cat. No. 10009305  
 Nuclear Extraction Protease Inhibitor Cocktail (100X) - Cat. No. 10009303  
 p53 Transcription Factor Assay Kit - Cat. No. 600020  
 p53 Designer Transcription Factor Assay Kit - Cat. No. 600030  
 PPAR $\alpha$  Transcription Factor Assay Kit - Cat. No. 10006915  
 PPAR $\delta$  Transcription Factor Assay Kit - Cat. No. 10006914  
 PPAR $\gamma$  Transcription Factor Assay Kit - Cat. No. 10006855  
 SREBP-1 Transcription Factor Assay Kit - Cat. No. 10010854  
 SREBP-2 Transcription Factor Assay Kit - Cat. No. 10007819

## Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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## NOTES

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