TransAM™
PPARγ Transcription Factor Assay Kits

(version E2)

Catalog Nos. 40196 & 40696

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Overview

Peroxisome proliferator-activated receptor γ (PPARγ) is a transcription factor that is activated in response to a high-fat diet. Besides its key role in lipid metabolism, PPARγ has been shown to be involved in chronic diseases such as diabetes, obesity, atherosclerosis and cancer. Therefore, accurate monitoring of PPARγ activation in cells, tissues or animals is crucial for biomedical research and drug development. To date, such research projects are tedious and time consuming, and lack high-throughput screening methods.

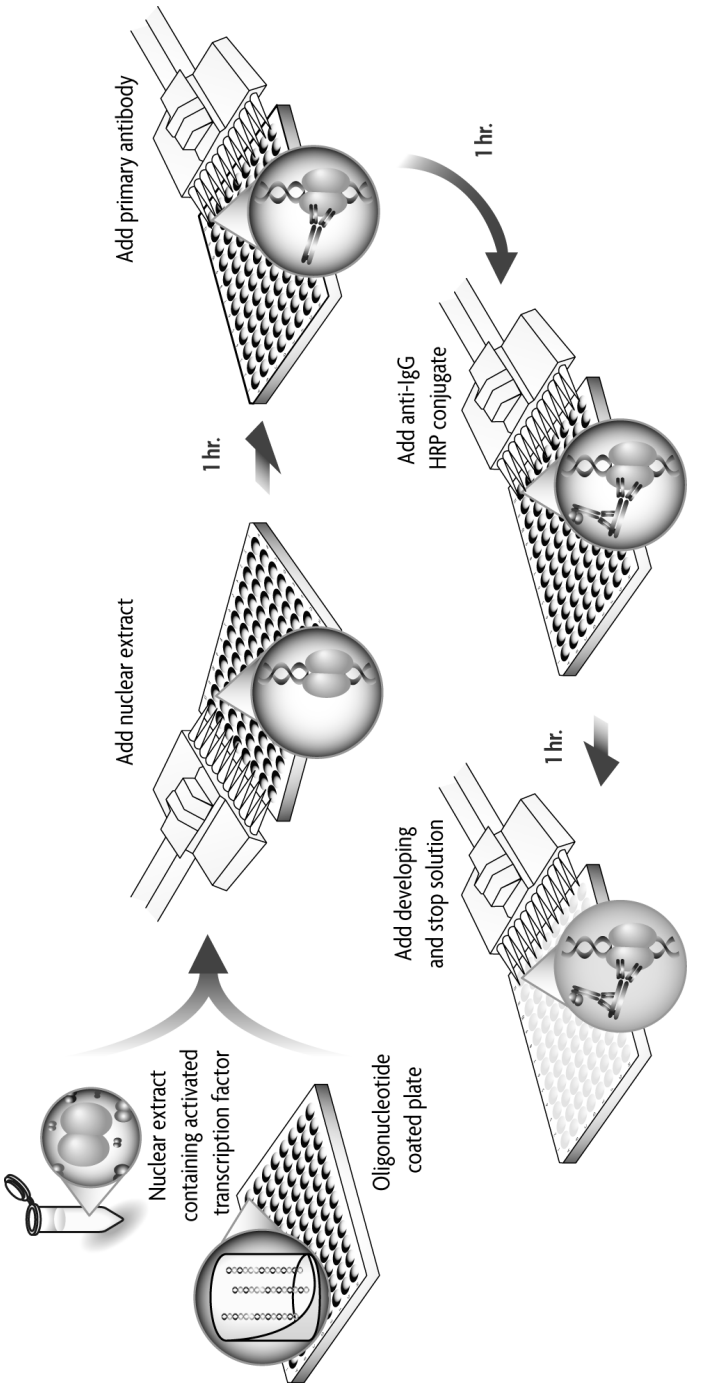
With its patented TransAM™ method*, Active Motif introduced the first ELISA-based kits to detect and quantify transcription factor activation. TransAM Kits combine a fast, user-friendly format with a sensitive, specific assay. TransAM PPARγ Kits are designed specifically for the study of PPARγ. They contain a 96-well plate to which oligonucleotide containing the peroxisome proliferator response element (PPRE) has been immobilized. PPARs contained in nuclear extracts bind specifically to this oligonucleotide and are detected through use of an antibody directed against PPARγ. Addition of a secondary antibody conjugated to horseradish peroxidase (HRP) provides sensitive colorimetric readout that is easily quantified by spectrophotometry. The 96-well plate with individual strips of 8 wells is suitable for manual use or for high-throughput screening applications. TransAM PPARγ Kits are available in two sizes:

product	format	catalog no.
TransAM PPARγ	1 x 96-well plate	40196
	5 x 96 well plates	40696

See Active Motif products related to the PPARγ transcription factor in Appendix, Section B.

* Technology covered by AAT-filed patents and licensed to Active Motif.

Flow Chart of Process



PPARγ Transcription Factor

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors involved in lipid transport and metabolism (see 1 for review). They form a subfamily of the nuclear receptor superfamily, along with the receptors for thyroid hormone, retinoic acid, vitamin D, ecdysone and the orphan receptors Rev-ErbAα and E75. Three PPAR isotypes have been identified: α, β (also called δ and NUC1) and γ. Their roles in chronic diseases such as diabetes, obesity, atherosclerosis and cancer are heavily investigated[2, 3]. PPARα and PPARγ contribute to atherosclerotic plaque formation by regulating plasma lipoprotein concentrations, affecting foam cell formation, modulating the inflammatory response and influencing plaque stability. PPARα acts as a global regulator of fatty acid catabolism. Recent studies have shown that PPARβ is also involved in lipid metabolism. PPARγ can modulate inflammation by reducing the production of inflammatory cytokines in monocytes[4, 5]. PPARγ may also promote atherosclerosis by stimulating the uptake of oxidized LDL that supports foam cell formation[6]. In contrast to PPARα, PPARγ promotes fat storage by increasing adipocyte differentiation and transcription of a number of lipogenic proteins.

PPARα is expressed mostly in brown adipose tissue and liver, then kidney, heart and skeletal muscle. PPARγ is mainly expressed in adipose tissue and, to a lesser extent, in the colon, the immune system and the retina. PPARβ is found in many tissues, but the highest expression is in the gut, kidney and heart.

PPARs are activated by natural ligands such as fatty acids and eicosanoids, and various synthetic ligands such as lipid-lowering fibrates and anti-diabetic glitazones[7, 8]. PPARs bind to peroxisome proliferator response elements (PPREs), which consist of a direct repeat of the nuclear receptor hexameric DNA core recognition motif spaced by one nucleotide (5′-AACTAGGNCAAAGGTCA-3′). PPARs bind only to PPREs as heterodimers with the receptor for 9-cis retinoic acid RXR (retinoid X receptor). The PPRE repeat structure imposes a polarity to the bound heterodimer[9]. PPAR interacts with the upstream extended core hexamer, whereas RXR occupies the downstream motif. PPAR-mediated transactivation results from the combination of PPAR:RXR binding to a PPRE and ligand activation of this complex. The conformational change of PPAR triggered by ligand binding, or by other activation processes such as phosphorylation, is believed to generate a transcriptionally active complex by forming specific contacts with coactivator proteins.

Transcription Factor Assays

To date, three methods are widely used to measure PPARγ activation, either directly or indirectly:

1. PPARγ activation can be determined by Western Blot by using antibodies specific for PPARγ protein. This method is time consuming (up to 2 days once the nuclear extracts are prepared), and is not suitable for processing large numbers of samples.

2. The DNA-binding capacity of PPARγ can be assayed by gel retardation, also called electrophoretic mobility shift assay (EMSA). In this method, nuclear extracts are incubated with a radioactive double-stranded oligonucleotide probe containing the consensus sequence

for PPAR binding. If PPARγ is active in the nuclear extract, it will bind to the probe. Samples are then resolved by electrophoresis on a native polyacrylamide gel, followed by autoradiography. This method is sensitive, but like the previous procedure, it is time consuming (multiple days of gel exposure may be required to achieve sufficient sensitivity) and it cannot be applied to high-throughput screening. Gelshift assays also require special precautions and equipment for handling radioactivity.

3. Another method used to assay PPARγ activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing a PPRE. The promoter can be artificial, made of several PPRE cis-elements and a TATA box, or natural, like the acylCoA oxidase promoter sequence. However, the procedure is limited by the following issues: (i) reporter gene assays have to be repeated several times to obtain statistically reliable data; and (ii) reporter gene assays are sensitive to confounding factors that may influence the expression level of the reporter gene, and therefore assays have to be carefully standardized. Reporter gene assays are sensitive and easy to perform with a large number of samples, but require efficient cell transfection with the reporter plasmid.

TransAM PPARγ

PPARγ is involved in fatty acid metabolism and intake by adipocytes, and therefore represents an excellent pharmacological target to develop drugs for treating obesity and diabetes. However, pharmaceutical research in this field has been hampered by the lack of convenient assays suitable for high sample number experiments.

To overcome this, Active Motif is introducing a high-throughput assay to quantify PPARγ activation[10]. The TransAM Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM PPARγ Kits contain a 96-well plate on which has been immobilized an oligonucleotide that contains a PPRE (5´-AACTAGGTCAAAGGTCA-3´). PPAR contained in nuclear extract specifically binds to this oligonucleotide. The primary antibody used in the TransAM PPARγ Kit recognizes an accessible epitope on PPARγ protein upon DNA binding. Addition of a secondary HRP-conjugated antibody provides a sensitive colorimetric readout easily quantified by spectrophotometry. Once the nuclear extracts are prepared, this assay is completed in less than 3.5 hours. As this assay is performed in 96-well plates, a large number of samples can be handled simultaneously, enabling high-throughput automation. This assay is specific for PPARγ activation and has been shown to be 5-fold more sensitive and 20-fold faster than the gel-retardation technique. With the 3.5-hour TransAM procedure, we could detect PPARγ activation using as little as 1.0 µg of nuclear extract. A comparable assay using EMSA required 5 µg of nuclear extract and a 3-day autoradiography.

TransAM has many applications including the study of drug potency toward PPARγ binding, PPARγ transcriptional activity regulation and protein structure/function studies of PPARγ and its cofactors in areas such as diabetes, obesity, atherosclerosis, carcinogenesis, and many more.

Kit Performance and Benefits

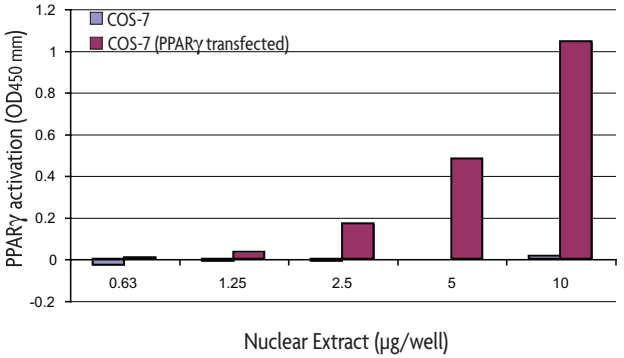
The TransAM PPARγ Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: > 1.0 µg nuclear extract/well. TransAM PPARγ is 5-fold more sensitive than EMSA.

Range of detection: TransAM provides quantitative results from 0.6 to 10 µg of nuclear extract/well (see graph below).

Cross-reactivity: TransAM PPARγ detects PPARγ$_1$ and PPARγ$_2$ from human, mouse and rat origins. It will not cross-react with PPARα or PPARd.

Assay time: 3.5 hours. TransAM is 20-fold faster than EMSA.



Monitoring PPAR activation with the TransAM PPARγ Kit: Different amounts of nuclear extracts from untransfected and PPARγ transfected COS-7 cells are tested for PPAR activation by using the TransAM PPARγ Kit. These results are provided for demonstration only.

Kit Components and Storage

Except for the nuclear extract that must be kept at -80ºC, kit components can be stored at -20ºC prior to first use. Then, we recommend storing each component at the temperature indicated in the table below.

Reagents	Quantity 1 plate / 5 plates	Storage / Stability
PPARγ antibody	12 µl /60 µl	-20°C for 6 months
Anti-mouse HRP-conjugated IgG	11 µl / 55 µl (0.2 mg/ml)	-20°C for 6 months
Wild-type oligonucleotide AM8	100 µl / 500 µl (20 pmol/µl)	-20°C for 6 months
Mutated oligonucleotide AM8	100 µl / 500 µl (20 pmol/µl)	-20°C for 6 months
COS-7 nuclear extract (PPARγ transfected)	16 µl / 80 µl (2.5 mg/ml)	-80°C for 6 months
Dithiothreitol (DTT) (1 M)	100 µl / 500 µl	-20°C for 6 months
Protease Inhibitor Cocktail	100 µl / 500 µl	-20°C for 6 months
Herring sperm DNA	100 µl / 500 µl (1 mg/ml)	-20°C for 6 months
Lysis Buffer AM1	10 ml / 50 ml	4°C for 6 months
Binding Buffer AM6	10 ml / 50 ml	4°C for 6 months
10X Wash Buffer AM2	22 ml / 110 ml	4°C for 6 monthsl
10X Antibody Binding Buffer AM2	2.2 ml / 11 ml	4°C for 6 months
Developing Solution	11 ml / 55 ml	4°C for 6 months
Stop Solution	11 ml / 55 ml	4°C for 6 months
96-well PPARγ assay plate	1 / 5	4°C for 6 months
Plate sealer	1 / 5	

Additional materials required

- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform
- Microplate spectrophotometer capable of reading at 450 nm (655 nm as optional reference wavelength)

For Nuclear Extract preparation

- Hypotonic Buffer
- Phosphatase Inhibitor Buffer
- 10X PBS
- Detergent (NP-40)

Buffer Preparation and Recommendations

Preparation of Complete Lysis Buffer

We provide an excess of Lysis Buffer AM1 in order to perform the assay AND to prepare customized nuclear extracts. Our Nuclear Extract Kit can also be purchased separately (Cat. Nos. 40010 & 40410). Prepare the amount of Complete Lysis Buffer required for the assay by adding 1 µl of 1 M DTT and 10 µl of Protease Inhibitor Cocktail per ml of Lysis Buffer AM1 (see the Quick Chart for Preparing Buffers in this section). Some of the protease inhibitors lose their activity after 24 hours once diluted. Therefore, we recommend using the Complete Lysis Buffer immediately for cell lysis. The remaining amount should be discarded if not used in the same day.

Preparation of Complete Binding Buffer

Prepare the amount of Complete Binding Buffer required for the assay by adding 10 µl of 1 mg/ml Herring sperm DNA per ml of Binding Buffer AM6 (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Complete Binding Buffer.

Preparation of 1X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 100 ml of 1X Wash Buffer required, dilute 10 ml 10X Wash Buffer AM2 with 90 ml distilled water (see the Quick Chart for Preparing Buffers in this section). Mix gently to avoid foaming. The 1X Wash Buffer may be stored at 4°C for one week. The Tween 20 contained in the 10X Wash Buffer AM2 may form clumps, therefore homogenize the buffer by incubating at 50°C for 2 minutes and mixing prior to use.

Preparation of 1X Antibody Binding Buffer

Prepare the amount of 1X Antibody Binding Buffer required for the assay as follows: For every 10 ml of 1X Antibody Binding Buffer required, dilute 1 ml 10X Antibody Binding Buffer AM2 with 9 ml distilled water (see the Quick Chart for Preparing Buffers in this section)*. Mix gently to avoid foaming. Discard remaining 1X Antibody Binding Buffer after use. The BSA contained in the 10X Antibody Binding Buffer AM2 may form clumps, therefore homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use. Dilute the primary antibody to 1:1000 and the HRP-conjugated secondary antibody to 1:1000 with the 1X Antibody Binding Buffer. Depending on the particular assay, the signal:noise ratio may be optimized by using higher dilutions of both antibodies. This may decrease the sensitivity of the assay.

* Volumes listed refer to the preparation of buffer for diluting both the primary & secondary antibodies.

Developing Solution

The Developing Solution should be warmed to room temperature before use. The Developing Solution is light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. The Developing Solution may develop a yellow hue over time. This does not affect product performance. A blue color present in the Developing Solution indicates that it has been contaminated and must be discarded. Prior to use, place the Developing Solution at room temperature for at least 1 hour. Transfer the amount of Developing Solution required for the assay into a secondary container before aliquoting into the wells (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Developing Solution.

Stop Solution

Prior to use, transfer the amount of Stop Solution required for the assay into a secondary container (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Stop Solution.

WARNING: The Stop Solution is corrosive. Wear personal protective equipment when handling, i.e. safety glasses, gloves and labcoat.

Nuclear extract

The COS-7 nuclear extract (PPARγ transfected) is provided as a positive control for PPARγ activation. Sufficient extract is supplied for 8 reactions per plate. This extract is optimized to give a strong signal when used at 5 µg/well. We recommend aliquoting the extract in 5 µl fractions and storing at -80ºC. Avoid multiple freeze/thaw cycles of the extract. Various cell extracts are available from Active Motif (see Appendix, Section B. Related Products).

Wild-type and mutated consensus oligonucleotides

The wild-type consensus oligonucleotide is provided as a competitor for PPARγ binding in order to monitor the specificity of the assay. Used at 40 pmol/well, the oligonucleotide will prevent PPARγ binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have little effect on PPARγ binding. Prepare the required amount of wild-type and/or mutated consensus oligonucleotide by adding 2 µl of appropriate oligonucleotide to 43 µl of Complete Binding Buffer per well being used (see the Quick Chart for Preparing Buffers in this section). To allow for optimum competition, add the oligonucleotide to the well prior to addition of the nuclear extract.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	For 1 well	For 1 strip (8 wells)	For 6 strips (48 wells)	For 12 strips (96 wells)
Complete Lysis Buffer	DTT	0.01 µl	0.1 µl	0.6 µl	1.2 µl
	Protease Inhibitor Cocktail	0.12 µl	0.9 µl	5.4 µl	10.8 µl
	Lysis Buffer AM1	11.12 µl	89 µl	534 µl	1.068 ml
	TOTAL REQUIRED	**11.25 µl**	**90 µl**	**540 µl**	**1.08 ml**
Complete Binding Buffer	Herring sperm DNA	0.45 µl	3.6 µl	21.6 µl	43.2 µl
	Binding Buffer AM6	44.5 µl	356 µl	2.138 ml	4.277 ml
	TOTAL REQUIRED	**45 µl**	**360 µl**	**2.16 ml**	**4.32 ml**
Complete Binding Buffer with wild-type or mutated oligonucleotide	Wild-type or mutated oligo	2 µl	16 µl	96 µl	N/A
	Complete Binding Buffer	43 µl	344 µl	2.064 ml	N/A
	TOTAL REQUIRED	**45 µl**	**360 µl**	**2.16 ml**	**N/A**
1X Wash Buffer	Distilled water	2.025 ml	16.2 ml	97.2 ml	194.4 ml
	10X Wash Buffer AM2	225 µl	1.8 ml	10.8 ml	21.6 ml
	TOTAL REQUIRED	**2.25 ml**	**18 ml**	**108 ml**	**216 ml**
1X Antibody Binding Buffer*	Distilled water	202.5 µl	1.62 ml	9.72 ml	19.44 ml
	10X Ab Binding Buffer AM2	22.5 µl	180 µl	1.08 ml	2.16 ml
	TOTAL REQUIRED	**225 µl**	**1.8 ml**	**10.8 ml**	**21.6 ml**
Developing Solution	**TOTAL REQUIRED**	**112.5 µl**	**900 µl**	**5.4 ml**	**10.8 ml**
Stop Solution	**TOTAL REQUIRED**	**112.5 µl**	**900 µl**	**5.4 ml**	**10.8 ml**

* Volumes listed refer to the preparation of buffer for diluting both the primary & secondary antibodies.

PPARγ Transcription Factor Assay

Determine the appropriate number of microwell strips required for testing samples, controls and blanks in duplicate. If less than 8 wells in a strip need to be used, cover the unused wells with a portion of the plate sealer while you perform the assay. The content of these wells is stable at room temperature if kept dry and, therefore, can be used later for a separate assay. Store the unused strips in the aluminum pouch at 4°C. Use the strip holder for the assay.

Prepare the Complete Lysis Buffer, Complete Binding Buffer, 1X Wash Buffer and 1X Antibody Binding Buffer as described above in the section Buffer Preparation and Recommendations. Multi-channel pipettor reservoirs may be used for dispensing the Complete Binding Buffer, Wash Buffer, Antibody Binding Buffer, Developing Solution and Stop Solution into the wells being used.

Step 1: Binding of PPARγ to its consensus sequence

1. Add 40 µl Complete Binding Buffer to each well to be used. If you wish to perform competitive binding experiments, add 40 µl Complete Binding Buffer containing 40 pmol (2 µl) of the wild-type or mutated consensus oligonucleotide (see the Buffer Preparation section above for a description of competitive binding).
2. **Sample wells:** Add 10 µl of sample diluted in Complete Lysis Buffer per well. We recommend using 2-10 µg of nuclear extract diluted in Complete Lysis Buffer per well. A protocol for preparing nuclear extracts can be found on page 11.

   Positive control wells: Add 5 µg of the provided nuclear extract diluted in 10 µl of Complete Lysis Buffer per well (2 µl of nuclear extract in 8 µl of Complete Lysis Buffer per well).

   Blank wells: Add 10 µl Complete Lysis Buffer only per well.
3. Use the provided adhesive cover to seal the plate. Incubate for 1 hour at room temperature with mild agitation (100 rpm on a rocking platform).
4. Wash each well 3 times with 200 µl 1X Wash Buffer. For each wash, flick the plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

Step 2: Binding of primary antibody

1. Add 100 µl diluted PPARγ antibody (1:1000 dilution in 1X Antibody Binding Buffer) to all wells being used.
2. Cover the plate and incubate for 1 hour at room temperature without agitation.
3. Wash the wells 3 times with 200 µl 1X Wash Buffer (as described in Step 1, No. 4).

Step 3: Binding of secondary antibody

1. Add 100 µl of diluted anti-mouse HRP-conjugated antibody (1:1000 dilution in 1X Antibody Binding Buffer) to all wells being used.
2. Cover the plate and incubate for 1 hour at room temperature without agitation.
3. During this incubation, place the Developing Solution at room temperature.
4. Wash the wells 4 times with 200 µl 1X Wash Buffer (as described in Step 1, No. 4).

Step 4: Colorimetric reaction

1. Add 100 µl Developing Solution to all wells being used.
2. Incubate 5-10 minutes at room temperature protected from direct light. Please read the Certificate of Analysis supplied with this kit for the optimal development time for this specific kit lot, which varies from lot to lot. Monitor the blue color development in the sample and positive control wells until it turns medium to dark blue. Do not overdevelop.
3. Add 100 µl Stop Solution. In presence of the acid, the blue color turns yellow.
4. Read absorbance on a spectrophotometer within 5 minutes at 450 nm with a reference wavelength of 655 nm. Blank the plate reader according to the manufacturer's instructions using the blank wells.

Preparation of Nuclear Extract

For your convenience, Active Motif offers a Nuclear Extract Kit (Cat. Nos. 40010 & 40410). This kit contains buffers optimized for use in TransAM Kits, which serves to reduce inconsistencies in the assay that may arise from using homemade or other buffers. If you prefer to make your own buffers, please refer to the following protocol.

This procedure can be used for a 15 ml cell suspension in a T75 flask. The yield is approximately 50 µg of nuclear proteins for 10^7 cells.

1. Collect 10 ml of cell suspension in a pre-chilled 15 ml tube.
2. Scrape the cells off the flask in the remaining 5 ml of media with a cell lifter. Transfer cells into the 15 ml tube and spin at 300 x g for 5 minutes at 4°C.
3. Discard supernatant. Resuspend cell pellet in 5 ml PBS/PIB and spin at 300 x g for 5 minutes at 4°C.
4. Discard supernatant. Resuspend the pellet in 1 ml ice-cold HB buffer by gentle pipetting and transfer the cells into a pre-chilled 1.5 ml tube.
5. Allow the cells to swell on ice for 15 minutes.
6. Add 50 µl 10% Nonidet P-40 (0.5 % final) and mix by gentle pipetting.
7. Centrifuge the homogenate for 30 seconds at 4°C in a microcentrifuge. Remove the supernatant (cytoplasmic fraction) and, if you wish to save this for other uses, transfer it into a pre-chilled microcentrifuge tube. (Store the cytoplasmic fraction at –80°C.)
8. Resuspend the nuclear pellet in 40 µl Complete Lysis Buffer and rock the tube gently on ice for 30 minutes on a shaking platform.
9. Centrifuge for 10 minutes at 14,000 x g at 4°C and save the supernatant (nuclear extract). Aliquot and store at –80°C. Avoid freeze/thaw cycles.
10. Determine the protein concentration of the extract by using a Bradford-based assay.

10X PBS	**For 250 ml, mix:**
0.1 M phosphate buffer, pH 7.5	3.55 g Na_2HPO_4 + 0.61 g KH_2PO_4
1.5 M NaCl	21.9 g
27 mM KCl	0.5 g

Adjust to 250 ml with distilled water. Prepare a 1X PBS solution by adding 10 ml 10X PBS to 90 ml distilled water. Sterilize the 1X PBS by filtering through a 0.2 µm filter. The 1X PBS is at pH 7.5. Store the filter-sterilized 1X PBS solution at 4°C.

PIB (Phosphatase Inhibitor Buffer)	For 10 ml, mix
125 mM NaF	52 mg
250 mM β-glycerophosphate	0.55 g
250 mM para-nitrophenyl phosphate (PNPP)	1.15 g
25 mM $NaVO_3$	31 mg

Adjust to 10 ml with distilled water. Mix the chemicals by vortexing. Incubate the solution at 50°C for 5 minutes. Mix again. Store at –20°C.

PBS/PIB

Prior to use, add 0.5 ml of PIB in 10 ml of 1X PBS.

HB (Hypotonic Buffer)	For 50 ml, mix
20 mM Hepes, pH 7.5	0.24 g
5 mM NaF	12 mg
10 µM Na_2MoO_4	5 µl of a 0.1 M solution
0.1 mM EDTA	10 µl of a 0.5 M solution

Adjust pH to 7.5 with 1 N NaOH. Adjust volume to 50 ml with distilled water. Sterilize by filtering through a 0.2 µm filter. Store the filter-sterilized solution at 4°C.

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Appendix

Section A. Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION
No signal or weak signal in all wells	Omission of key reagent	Check that all reagents have been added in the correct order
	Substrate or conjugate is no longer active	Test conjugate and substrate for activity
	Enzyme inhibitor present	Sodium azide will inhibit the peroxidase reaction, follow our recommendations to prepare buffers
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader
	Incorrect assay temperature	Bring substrate to room temperature
	Inadequate volume of Developing Solution	Check to make sure that correct volume is delivered by pipette
High background in all wells	Developing time too long	Stop enzymatic reaction as soon as the positive wells turn medium-dark blue
	Concentration of antibodies too high	Increase antibody dilutions
	Inadequate washing	Ensure all wells are filled with Wash Buffer and follow washing recommendations
Uneven color development	Incomplete washing of wells and follow washing recommendations	Ensure all wells are filled with Wash Buffer
	Well cross-contamination	Follow washing recommendations
High background in sample wells	Too much nuclear extract per well	Decrease amount of nuclear extract down to 1-2 µg/well
	Concentration of antibodies too high	Perform antibody titration to determine optimal working concentration. Start using 1:500 for primary antibody and 1:5000 for the secondary antibody. The sensitivity of the assay will be decreased
No signal or weak signal in sample wells	Not enough nuclear extract per well	Increase amount of nuclear extract, not to exceed 40 µg/well
	PPARγ is poorly activated or inactivated in nuclear fractions	Perform a time course for PPARγ activation in the studied cell line
	Nuclear extracts are not from rat, mouse or human origin	Perform study with a human, mouse or rat model

Technical Services

If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

Active Motif North America

Toll free: 877.222.9543
Direct: 760.431.1263
Fax: 760.431.1351
E-mail: tech_service@activemotif.com

Active Motif Europe

UK Free Phone: 0800/169 31 47
France Free Phone: 0800/90 99 79
Germany Free Phone: 0800/181 99 10
Direct: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan

Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

Direct: (86)-21-20926090
Cell Phone: 18521362870
E-mail: techchina@activemotif.com

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