TransAM™

MyoD Transcription Factor Assay Kits

(version B2)

Catalog Nos. 47196 & 47696

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TABLE OF CONTENTS

Page

Overview 1

Flow Chart of Process 2

Introduction

MyoD Transcription Factor 3

Transcription Factor Assays 3

TransAM MyoD 4

Kit Performance and Benefits 5

Kit Components and Storage 6

Additional Materials Required 7

Protocols

Buffer Preparation and Recommendations 7

Quick Chart for Preparing Buffers 9

MyoD Transcription Factor Assay 9

Preparation of Nuclear Extract 11

References 12

Appendix

Section A. Troubleshooting Guide 13

Section B. Related Products. 14

Technical Services 15

Overview

The MyoD transcription factor is the central target in the signaling pathways that regulate muscle development. Although MyoD family members have been studied for decades in several muscle cell systems, the overall muscle differentiation program is still a strong area of focus to understand muscle decay. Because age and wound-induced muscle wasting, cachexia and myopathies are still incurable, accurate monitoring of MyoD activation in cells, tissues and 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.

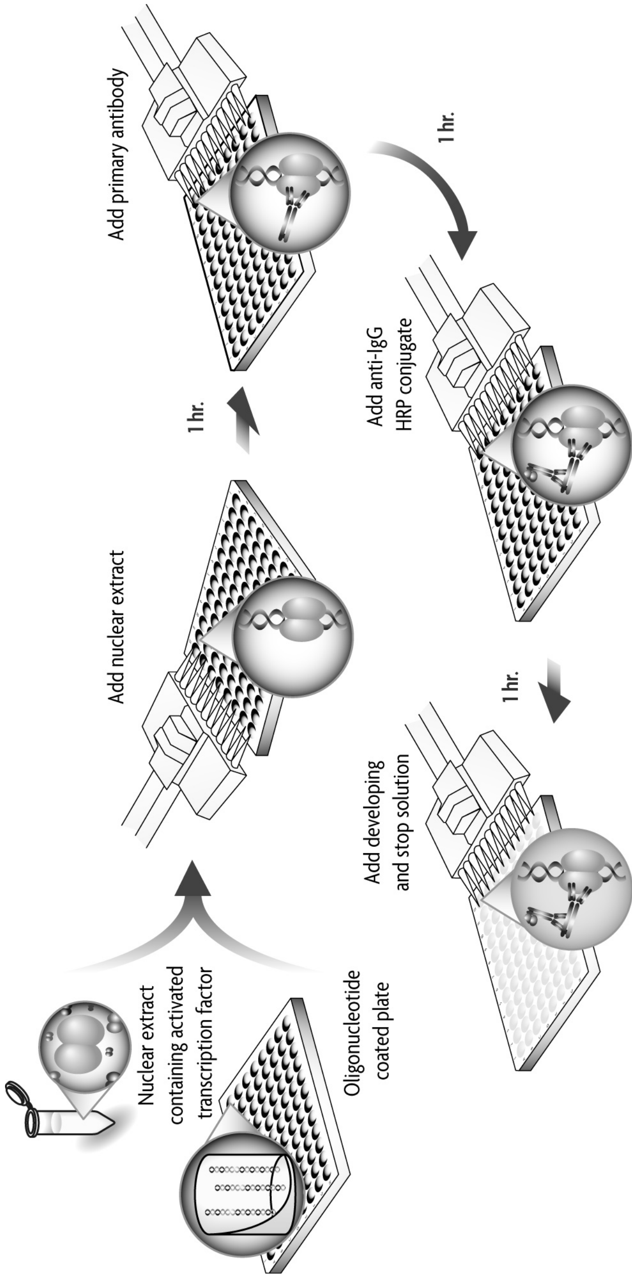
With its patented TransAM™ method*, Active Motif introduced the first ELISA-based kits to detect and quantify transcription factor activation. TransAM Kits combine a fast, user-friendly format with a sensitive and specific assay. TransAM MyoD Kits are designed specifically for the study of MyoD. They contain a 96-well plate to which oligonucleotide containing an E-box has been immobilized. MyoD complexes contained in nuclear extracts bind specifically to this oligonucleotide and are detected through the use of an antibody directed against MyoD. 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 MyoD Kits are available in two sizes:

product	format	catalog no.
TransAM MyoD	1 x 96-well plate 5 x 96 well plates	47196 47696

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

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

Flow Chart of Process



Introduction

MyoD Transcription Factor

The Myogenic Regulatory Factor (MRF) family is composed of 4 members: Myf-3 (MyoD), Myf-4 (myogenin), Myf-5 and Myf-6 (MRF4/herculin). These proteins are involved in the skeletal muscle differentiation program that balances myoblast proliferation vs. differentiation (reviewed in 1). Activation of this program in response to growth factor withdrawal is accompanied by myoblast cell fusion to form multinucleated myotubes, irreversible commitment of muscle cells to the post-mitotic state, and transcriptional activation of muscle-specific genes[2]. MyoD is expressed in undifferentiated myogenic proliferating cells. Expression of MyoD family genes activates myogenesis in various non-muscle cell types[3]. In mice bearing a null Myf-5/MyoD double mutation, muscle fibers and myoblasts are absent. MyoD acts downstream of Pax-3 and Myf-5 in skeletal myogenesis[4] and is sensitive to growth factors and transforming gene products such as Ras and Fos, which have been shown to abolish the expression of MyoD in myoblasts. MyoD induces muscle-specific alternative splicing of mouse mitochondrial ATP synthase gamma-subunit, beta-tropomyosin, MEF2A and MEF2D pre-mRNAs.

MyoD is a transcription factor with a basic helix-loop-helix (bHLH) DNA-binding domain that mediates dimerization and binding to a DNA consensus sequence termed an E-box (CANNTG)[5]. These E-boxes are present in the control regions of several skeletal muscle-specific genes such as the muscle creatinine kinase, myosin light chains 1 and 3, desmin and the acetylcholine receptor. Its dimeric partners are E-box binding proteins such as Tal-1, E12 and E47[6]. While MyoD and E47 both form homodimeric complexes with DNA that have similar dissociation constants, a stochiometric mixture of these bHLH domains forms almost exclusively heterodimeric complexes on DNA[7]. The DNA binding activity of MyoD is repressed by forming heteromeric complexes with inhibitory proteins, such as Id protein and Mist1 transcription factor, which occupy the specific E-box target sites[8, 9].

Transcription Factor Assays

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

1. MyoD activation can be determined by Western Blot by using antibodies specific for MyoD 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 MyoD can be assayed by gel retardation, also called electrophoretic mobility shift assay (EMSA). In this case, nuclear extracts are incubated with a radioactive double-stranded oligonucleotide probe containing the consensus sequence for MyoD binding. If MyoD 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 largely used method to assay MyoD activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing an E-box. The promoter can be artificial, made of several E-boxes and a TATA box, or natural, like the creatine kinase 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 MyoD

MyoD is the key regulator of myogenesis. Besides the physiological activation of myogenic factors during development, recent studies have shown that cytokines such as TNF-α and IFN-γ regulate *myo*D gene expression. NFκB and NFAT signaling pathways are activated during muscle differentiation, regeneration and decay in AIDS and cancer patients. Therefore, known drugs can be applied in muscle-related therapeutic areas. However, this field has been hampered by the lack of convenient assays suitable for monitoring the activation of MyoD family members and for high sample number experiments.

To overcome this, Active Motif is introducing a high-throughput assay to quantify MyoD activation[10]. TransAM Kits combine a fast, user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM MyoD Kits contain a 96-well plate on which has been immobilized an oligonucleotide containing an E-box (5´-CACCTG-3´). MyoD contained in nuclear extract specifically binds to this oligonucleotide. The primary antibody used in TransAM MyoD Kits recognizes an epitope on MyoD protein that is accessible 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 the assay is performed in 96-well plates, a large number of samples can be handled simultaneously, enabling high-throughput automation. The assay is specific for MyoD activation and has been shown to be more sensitive and faster than the gel-retardation technique. With the 3.5-hour TransAM procedure, we could detect MyoD activation with as little as 0.15 µg of nuclear extract from confluent C_2C_{12} mouse myoblast cells. A comparative assay using EMSA required 10 µg of nuclear extract and a 7-day autoradiography.

TransAM MyoD has many applications including the study of transcriptional regulation during muscle development, cell fusion and MyoD antagonist drug validation in areas such as cancer, cachexia, myopathies and many more.

Kit Performance and Benefits

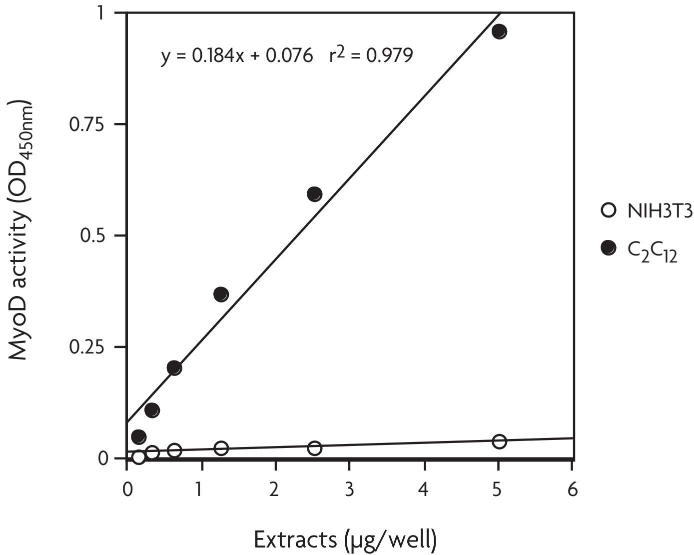
The TransAM MyoD Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: < 0.15 µg nuclear extract/well. TransAM MyoD is 60-fold more sensitive than EMSA.

Range of detection: TransAM provides quantitative results within a 0.25-5 µg nuclear extract/well range (see graph below).

Cross-reactivity: TransAM MyoD detects MyoD in human, mouse and rat extracts.

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



Monitoring MyoD activity with the TransAM MyoD Kit: Increasing amounts of nuclear extracts from confluent C_2C_{12} cells (filled circles) and from NIH3T3 cells (open circles) are tested for MyoD activity using the TransAM MyoD Kit. These curves are provided for demonstration only.

Kit Components and Storage

The nuclear extract must be kept at -80°C. Other Kit components can be stored at -20°C 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
MyoD antibody	11 µl / 55 µl (0.2 mg/ml)	4°C for 6 months
Anti-rabbit HRP-conj. IgG	11 µl / 55 µl (0.4 mg/ml)	4°C for 6 months
Wild-type oligonucleotide AM17	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
MyoD mutated oligonucleotide AM17	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
C_2C_{12} nuclear extract	40 µl / 200 µl (2.5 mg/ml)	-80°C for 6 months
Dithiothreitol (DTT)	100 µl / 500 µl (1 M)	-20°C for 6 months
Protease Inhibitor Cocktail	100 µl / 500 µl	-20°C for 6 months\
Lysis Buffer AM1	10 ml / 50 ml	4°C for 6 months
Binding Buffer AM4	10 ml / 50 ml	4°C for 6 months
10X Wash Buffer AM2	22 ml / 110 ml	4°C for 6 months
10X Antibody Binding Buffer AM3	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 MyoD 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). The presence of phosphatase inhibitors gives a yellow coloration to Lysis Buffer AM1. Prepare the amount of Complete Lysis Buffer required for the assay (see the Quick Chart for Preparing Buffers in this section) by adding 1 µl 1 M DTT and 10 µl Protease Inhibitor Cocktail per ml of Lysis Buffer AM1. 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.

Binding Buffer

This is supplied ready-to-use.

Preparation of 1X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay (see the Quick Chart for Preparing Buffers in this section) as follows: For every 100 ml of 1X Wash Buffer required, dilute 10 ml 10X Wash Buffer AM2 with 90 ml of distilled water. 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 (see the Quick Chart for Preparing Buffers in this section*) as follows: For every 10 ml of 1X Antibody Binding Buffer required, dilute 1 ml 10X Antibody Binding Buffer AM3 with 9 ml of distilled water. Mix gently to avoid foaming. Discard remaining 1X Antibody Binding Buffer after use. The BSA contained in the 10X Antibody Binding Buffer AM3 may form clumps, therefore homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use. Dilute both the primary and the HRP-conjugated secondary antibodies 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 the 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 (see the Quick Chart for Preparing Buffers in this section) into a secondary container. 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.

C_2C_{12} nuclear cell extract

The C_2C_{12} nuclear extract is provided as a positive control for MyoD activation. Sufficient extract is supplied for 20 reactions per plate. This extract is optimized to give a strong signal when used at 5 µg/well. We recommend aliquoting the extract in 10 µl fractions and storing at -80°C. 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 MyoD binding in order to monitor the specificity of the assay. Used at 20 pmol/well, the oligonucleotide will prevent MyoD binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no effect on MyoD binding. Prepare the required amount of wild-type and/or mutated consensus oligonucleotide by adding 2 µl of the appropriate oligonucleotide to 43 µl Binding Buffer AM4 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**
Binding Buffer AM4	**TOTAL REQUIRED**	**45 µl**	**360 µl**	**2.16 ml**	**4.32 ml**
Binding Buffer with wild-type or mutated oligonucleotide	Wild-type or mutated oligo	2 µl	16 µl	2.4 µl	N/A
	Binding Buffer AM4	43 µl	344 µl	2.158 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	225.09 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 AM3	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.

MyoD 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 the assay is performed. 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, 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 Binding Buffer, Wash Buffer, Antibody Binding Buffer, Developing Solution and Stop Solution into the wells being used.

Step 1: Binding of MyoD to its consensus sequence

1. Add 40 µl Binding Buffer AM4 to each well to be used. If you wish to perform competitive binding experiments, add 40 µl Binding Buffer AM4 that contains 20 pmol (2 µl) of the wild-type or mutated 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 C_2C_{12} nuclear extract diluted in 10 µl Complete Lysis Buffer per well (2 µl of extract in 8 µl 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 of diluted MyoD 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 above in Step 1, No. 4.

Step 3: Binding of secondary antibody

1. Add 100 µl of diluted anti-rabbit 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 above in Step 1, No. 4.

Step 4: Colorimetric reaction

1. Add 100 µl Developing Solution to all wells being used.
2. Incubate 5 min 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 by 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 confluent cell layer of 75 cm^2 (100-mm dish). The yield is approximately 0.15 mg of nuclear proteins for 9 x 10^6 cells.

1. Wash cells with 10 ml ice-cold PBS/PIB. Discard PBS/PIB.
2. Add 10 ml ice-cold PBS/PIB and scrape the cells off the dish with a cell lifter. Transfer cells into a pre-chilled 15 ml tube and spin at 300 x *g* for 5 minutes at 4°C.
3. 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.
4. Allow the cells to swell on ice for 15 minutes.
5. Add 50 µl 10% Nonidet P-40 (0.5 % final) and vortex the tube vigorously for 10 seconds.
6. 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.)
7. Resuspend the nuclear pellet in 50 µl Complete Lysis Buffer and rock the tube gently on ice for 30 minutes on a shaking platform.
8. 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.
9. 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 PIB to 10 ml 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	Ensure all wells are filled with Wash Buffer and follow washing recommendations
	Well cross-contamination	Follow washing recommendations
High background in sample wells	Too much nuclear cell 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 1:1000 for primary antibody and 1:5000 for the secondary antibody. The sensitivity of the assay will be decreased
No signal or weak signal	Not enough nuclear extract per well	Increase amount of nuclear extract not to exceed 40 µg per well
	MyoD is poorly activated or inactivated in nuclear fraction	Perform a time course for MyoD activation in the studied cell line. Follow guidelines for nuclear extract preparation
	Extracts are not from human, mouse or rat origin	Check inter-species MyoD antibody cross-reactivity

Section B. Related Products

TransAM™ Kits	Unit	Catalog No.
NFκB p65	1 x 96-well plate 5 x 96-well plates	40096 40596
NFκB p50	1 x 96-well plate 5 x 96-well plates	41096 41596
CREB	1 x 96-well plate 5 x 96-well plates	42096 42596
pCREB	1 x 96-well plate 5 x 96-well plates	43096 43596
AP-1 c-Fos	1 x 96-well plate 5 x 96-well plates	44096 44596
AP-1 FosB	1 x 96-well plate 5 x 96-well plates	45096 45596
AP-1 c-Jun	1 x 96-well plate 5 x 96-well plates	46096 46596
HIF-1	1 x 96-well plate 5 x 96-well plates	47069 47596
PPARγ	1 x 96-well plate 5 x 96-well plates	40196 40696
p53	1 x 96-well plate 5 x 96-well plates	41196 41696
Sp1	1 x 96-well plate 5 x 96-well plates	41296 41796
NFATc1	1 x 96-well plate 5 x 96-well plates	47196 47696

Cell extracts

HeLa whole-cell extract	200 µg	40050
HeLa whole-cell extract (IL-1α-stimulated)	200 µg	40100
HeLa whole-cell extract (TNF-α-stimulated)	200 µg	40200
HeLa nuclear extract (TNF-α-stimulated)	200 µg	40210
WI-38 nuclear extract (Forskolin-stimulated)	200 µg	40300
PC-12 nuclear extract (Forskolin-stimulated)	200 µg	40400
WI-38 nuclear extract (PMA-stimulated)	200 µg	40500
COS-7 nuclear extract ($CoCl_2$-treated)	200 µg	40600
THP-1 nuclear extract (PMA-stimulated)	200 µg	40700
MCF-7 nuclear extract (H_2O_2-treated)	200 µg	40800
C_2C_{12} nuclear extract (undifferentiated)	200 µg	36005
C_2C_{12} nuclear extract (differentiated)	200 µg	36078

Related Kits

Nuclear Extract Kit	100 rxn 400 rxn	40010 40410

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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