TransAM™ IRF-3 (Human)

(version A3)

Catalog Nos. 48396 & 48896

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Overview

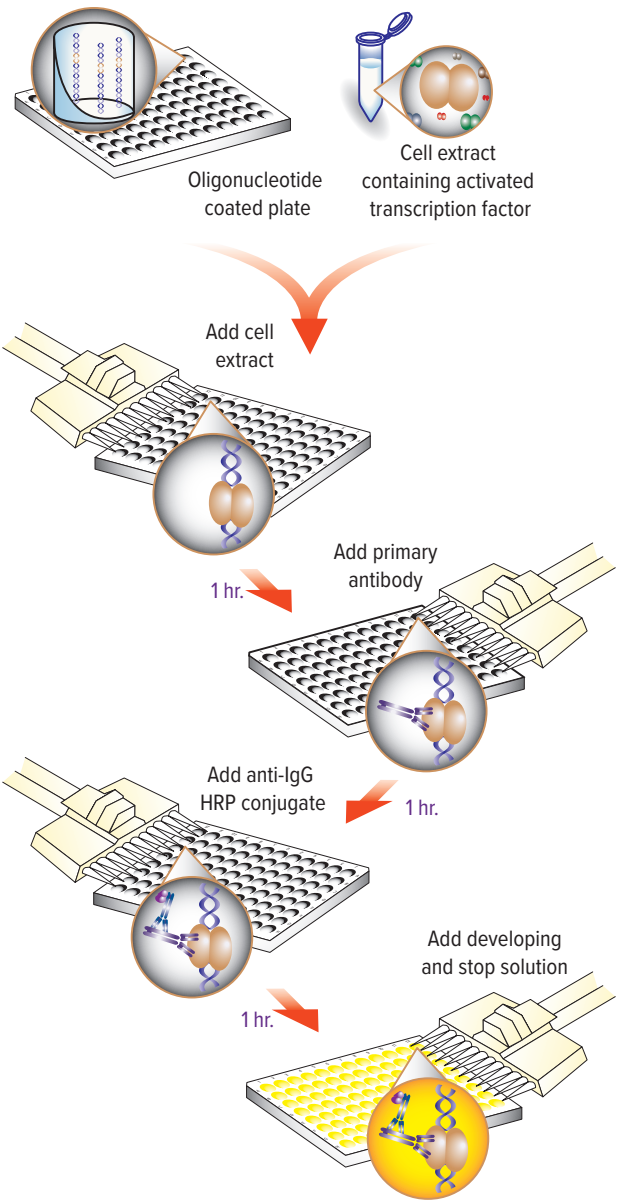
Interferon Regulatory Factors (IRFs) constitute a family of transcription factors that are involved in host defense via regulation of anti-viral immune responses, cell growth and hematopoietic development. Therefore, accurate monitoring of IRF activation in cells, tissues and animals is crucial for many biomedical research and drug development projects. 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 IRF-3 Kits are designed specifically to detect and quantify IRF-3 activation. They contain a 96-well plate to which oligonucleotide containing an interferon regulatory factor element (IRFE) has been immobilized. IRF-3 contained in nuclear extract binds specifically to this oligonucleotide and is detected through use of an antibody directed against IRF-3. Addition of a secondary antibody conjugated to horseradish peroxidase (HRP) provides a 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.

product	format	catalog no.
TransAM™ IRF-3 (Human)	1 x 96 rxns	48396
TransAM™ IRF-3 (Human)	5 x 96 rxns	48896

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

Flow Chart of Process



Introduction

IRF Transcription Factor

The interferon (IFN) regulatory factor (IRF) family is a group of transcription factors that have extensive homology in their DNA-binding domain (DBD)[1]. The many members of the IRF family are involved in the regulation of interferon (IFN) a and b and play a role in host anti-viral immune regulation, cell growth and hematopoietic development[2]. The N-terminal binding domain of IRFs, the distinct feature of the family, is a modified helix-turn-helix characterized by repeated tryptophan residues separated by 10 to 18 amino acids[3]. All IRFs, except IRF-1 and IRF-2, have an IRF association domain (IAD) that is responsible for the interaction with other family members or with transcription factors such as PU.1, E47 and STAT. Another association domain (IAD2), present only in IRF-1 and IRF-2, is important for interaction with IRF-8. A nuclear localization signal has been identified in IRF-1, and similar sequences may also be present in other family members[4]. A bipartite nuclear retention signal located within the N-terminus of the DBD has been identified in

IRF-4, IRF-8 and IRF-9. IRFs also possess a transactivation domain in the middle of the protein.

IRFs bind DNA as dimers on sequences such as the IFN-stimulated response element (ISRE), AGTTTCNNCNY, the IFN consensus sequence (ICS), R(G/C)TTTC, or the IFN-regulatory factor element (IRFE), G(A)AAA(G/C)YGAAA(G/C)Y. While IRF-3 is constitutively expressed, the expression of other IRFs is induced by such stimuli as type I and II IFN, double-stranded RNA or the presence of viral components, which can also induce the activity of the IRF factors after they have been synthesized. IRF factors can cooperate with other factors with neighboring binding sites on promoters. For example, the IFN-β promoter provides the stepping stone for the formation of an "enhanceosome" containing ATF-2, c-Jun, IRF-3, NFκB and CBP.

In mammals, ten IRF family members have been identified[5]. IRF-1, an activator, is involved in mature lymphocyte apoptosis after DNA-damage. IRF-2 is mainly a repressor, but can also play a role in histone H4 expression. Both IRF-1 and IRF-2 interact with the co-activator P/CAF. IRF-1 has been described as a tumor suppressor and IRF-2 as a proto-oncogene. IRF-3 is a component of DRAF, a complex containing the acetylase CBP/p300, which binds ISRE-like sequences. Double-stranded RNA (dsRNA) or poly (I-C), a synthetic form of dsRNA, elicits IRF3 activation[6]. IRF-4 is required for the function and homeostasis of B and T-cells and can also interact with the Ets-family member PU.1 and with the E47 form of E2A. IRF-7 helps induce the IFN-α gene and repress the EBNA-1 gene from the Epstein-Barr virus. The expression of IRF-7 is induced by type I IFN, which is important for amplification of the signaling pathway[7]. IRF-8 (ICSBP) can interact with IRF-1 and 2, but primarily acts as a repressor. IRF-9 (ISGF3γ/p48) is part of the ISGF3 transcription factor, together with STAT1 and STAT2. IRF-10 functions in the late stages of anti-viral defense by regulating IFNγ target genes.

Transcription Factor Assays

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

1. IRF activation can be measured by native Western blot to observe IRF-3 dimers by using antibodies specific for IRF proteins. 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 IRF 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 IRF binding. If IRF 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 IRF activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing the IRF consensus sequence. The promoter can be artificial, made of several IRF cis-elements and a TATA box, or natural, like the GAAANN purine-rich virus response element (VRE) in the IFNA promoter[8]. 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. Therefore, assays have to be carefully standardized. This method is sensitive and easy to perform with a large number of samples but requires efficient cell transfection with the reporter plasmid.

TransAM IRF-3

The family of interferon regulatory factor (IRF) transcription factors play an important role in the regulation of interferons in response to infection by virus and in the regulation of interferon-inducible genes. As the function and activity of individual IRFs can depend both on their activation state as well as that of other IRF family members, the ability to simultaneously profile the activation states of different IRF family members is critical to understanding their role in the regulation of immune function and oncogenesis.

To help achieve this, Active Motif offers high-throughput assays to quantify activated IRF-3 and IRF-7[9]. The TransAM Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM IRF-3 Kits contain a 96-well plate on which has been immobilized oligonucleotide containing the IRF consensus binding site (5´-GAAACTGAAACT-3´). The active form of IRF-3 contained in nuclear extract specifically binds to this oligonucleotide. The primary antibody used to detect IRF recognizes an epitope on IRF-3 protein upon DNA binding. Addition of an HRP-conjugated secondary 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 IRF-3 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 procedure of TransAM, we could detect IRF activation using as little as 0.5 µg of nuclear extract.

TransAM has many applications including the study of drug potency, inhibitor or activator proteins, and/or protein structure/function in the IRF signaling pathway.

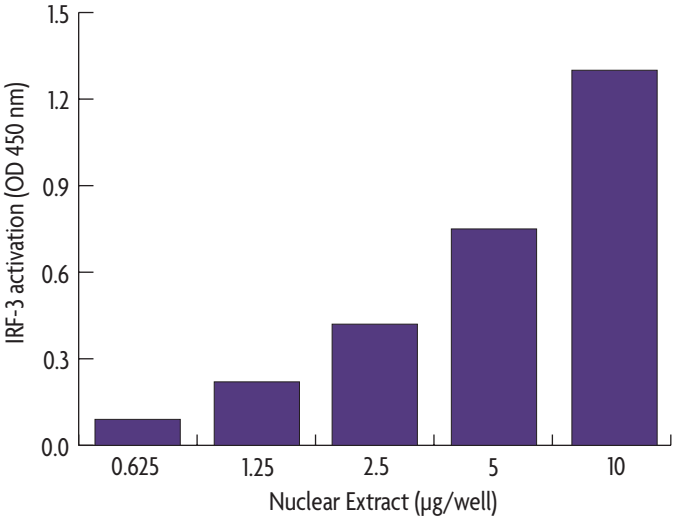
Kit Performance and Benefits

Detection limit: < 0.5 µg nuclear extract/well.

Range of detection: TransAM provides quantitative results from 0.5 to 10 µg of nuclear extract per well.

Cross-reactivity: TransAM IRF-3 specifically detects IRF-3 from human origin. For a TransAM IRF-3 kit that is specific for mouse and rat, please see catalog numbers 48496 and 48996.

Assay time: 3.5 hours.



Monitoring IRF-3 activation with the TransAM IRF-3 (Human) Kit:

0.625 to 10 µg of Cos-7 (poly I-C treated, 2 hr) nuclear extract were assayed per well. Data shown are the results from wells assayed in duplicate.

Kit Components and Storage

TransAM IRF-3 Kits are for research use only. Not for use in diagnostic procedures. 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. Avoid repeated freezing and thawing of the IRF-3 antibody. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity 1 plate / 5 plates	Storage
IRF-3 antibody (human)	10 µl / 25 µl	4°C
anti-mouse HRP-conjugated antibody	10 µl / 50 µl	4°C
Wild-type oligonucleotide AM6	100 µl / 500 µl (10 pmol/µl)	-20°C
Mutated oligonucleotide AM16	100 µl / 500 µl (10 pmol/µl)	-20°C
Cos-7 nuclear extract (Poly (I-C) 2 hr)	40 µl / 200 µl (2.5 µg/µl)	-80°C
Dithiothreitol (DTT) (1 M)	100 µl / 500 µl	-20°C
Protease Inhibitor Cocktail	100 µl / 500 µl	-20°C
Poly [d(I-C)]	100 µl / 500 µl	-20°C
Lysis Buffer AM1	10 ml / 50 ml	4°C
Binding Buffer AM2	10 ml / 50 ml	4°C
10X Wash Buffer AM2	22 ml / 110 ml	4°C
10X Antibody Binding Buffer AM3	2.2 ml / 11 ml	4°C
Developing Solution	11 ml / 55 ml	4°C
Stop Solution	11 ml / 55 ml	4°C
96-well IRF-3 assay plate	1 / 5	
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)

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. Please refer to the Appendix Section A for a protocol to prepare a nuclear extract. Our Nuclear Extract Kit can also be purchased separately (Cat. Nos. 40010 & 40410). Lysis Buffer AM1 contains phosphatase inhibitors to prevent dephosphorylation of IRF during the extract preparation and the assay. The presence of these inhibitors gives a yellow coloration to Lysis Buffer AM1. Prepare the amount of Complete Lysis Buffer required for the assay by adding 1 µl of 1 M DTT and 10 µl 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 1 µl of 1 M DTT and 10 µl of 17 µg/ml poly[d(I-C)] per ml of Binding Buffer AM2 (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 AM3 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 AM3 may form clumps, therefore homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use. Dilute the primary antibody with the 1X Antibody Binding Buffer to 1:1000 for the IRF-3 and the HRP-conjugated secondary antibodies. 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.

Cos-7 nuclear extract (Poly (I-C) 2 hr)

The Cos-7 (Poly (I-C) 2 hr) nuclear extract is provided as a positive control for IRF-3 activation. Sufficient extract is supplied for 10 reactions if using 10 µg per well. This extract is optimized to give a strong signal when used at 10 µg/well. We recommend aliquoting the extract in 5 µl fractions and storing at -80ºC. Avoid multiple freeze/thaw cycles of the extract.

Wild-type and mutated consensus oligonucleotides

The wild-type consensus oligonucleotide is provided as a competitor for IRF binding in order to monitor the specificity of the assay. Used at 20 pmol/well, the oligonucleotide will prevent IRF binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no inhibitory effect on IRF 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	1 well	1 strip (8 wells)	6 strips (48 wells)	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	11.12 µl	89.0 µl	534.0 µl	1.07 ml
	Total Required	**11.25 µl**	**90.0 µl**	**540.0 µl**	**1.08 ml**
Complete Binding Buffer	DTT	0.04 µl	0.3 µl	2.16 µl	4.3 µl
	Poly [d(I-C)]	0.45 µl	3.6 µl	21.6 µl	43.2 µl
	Binding Buffer	44.5 µl	356.1 µl	2.14 ml	4.27 ml
	Total Required	**45 µl**	**360 µl**	**2.16 ml**	**4.32 ml**
Binding Buffer with IRF wt or mut oligont	wt or mut oligont	2.0 µl	16.0 µl	96.0 µl	N/A
	Complete Binding Buffer	43.0 µl	344.0 µl	2.064 µl	N/A
	Total Required	**45.0 µl**	**360.0 µl**	**2.16 µl**	**N/A**
1X Washing Buffer	Distilled Water	2.025 ml	16.2 ml	97.2 ml	194.4 ml
	10X Washing Buffer	225.0 µl	1.8 ml	10.8 ml	21.6 ml
	Total Required	**2.25 ml**	**18.0 ml**	**108.0 ml**	**216.0 ml**
1X Antibody Binding Buffer*	Distilled Water	202.5 µl	1.62 ml	9.72 ml	19.44 ml
	10X Antibody Binding Buffer	22.5 µl	180.0 µl	1.08 ml	2.16 ml
	Total Required	**225.0 µl**	**1.8 ml**	**10.8 ml**	**21.6 ml**
Developing Solution	**Total Required**	**112.5 µl**	**900.0 µl**	**5.4 ml**	**10.8 ml**
Stop Solution	**Total Required**	**112.5 µl**	**900.0 µl**	**5.4 ml**	**10.8 ml**

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

IRF-3 (Human) 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 IRF 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 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 is provided on page 11.

   Positive control wells: Add 10 µg of the provided nuclear extract diluted in 10 µl of Complete Lysis Buffer per well (4 µl of nuclear extract in 6 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 IRF-3 antibody (1:1000 dilution in 1X Antibody Binding Buffer) to each well 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 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 2-15 minutes at room temperature protected from direct light. 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.

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Appendix

Section A. 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.

Preparation of Buffers for Nuclear Extract

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 p-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.

Section B: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in all wells 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 is 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 extract per well	Decrease amount of nuclear extract down to 1-2 µg/well
	Concentration of antibodies is 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 in sample wells	Not enough nuclear extract per well	Increase amount of nuclear extract to 50 µg/well
	IRF is poorly activated or inactivated in nuclear fractions	Perform a time course for IRF activation in the studied cell line
	Nuclear extracts are not from correct species	

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

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