TransAM™

HNF-1

Transcription Factor Assay Kits

(version B2)

Catalog Nos. 46196 & 46696

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Overview

Hepatocyte Nuclear Factor-1 (HNF-1) is a transcription factor that regulates genes in the liver, kidney, small intestine and thymus.[1] HNF-1 is critical for proper metabolism and secretion of pancreatic B cells; mutations in HNF-1 can lead to an early onset of type II diabetes. Therefore, accurate monitoring of HNF-1 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.

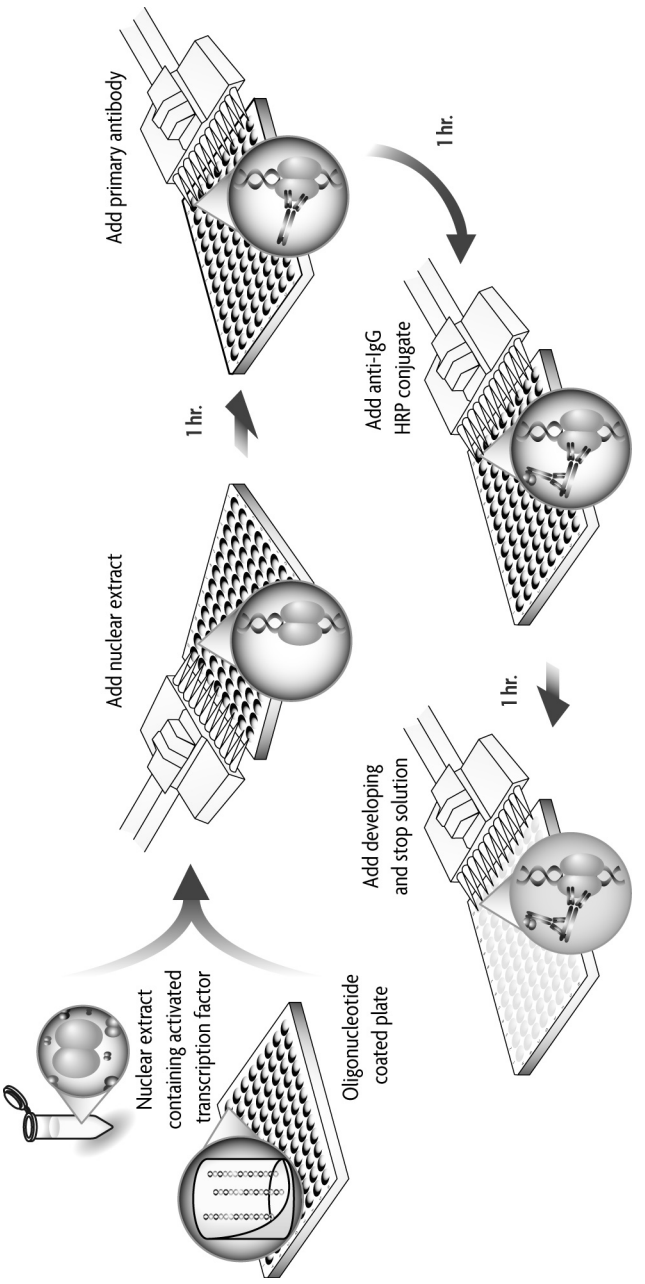
With its patented TransAM™ method*, Active Motif introduced the first ELISA-based kits to detect and quantify transcription factor activation. TransAM Kits combine a fast, user-friendly format with a sensitive, specific assay. TransAM HNF-1 Kits are designed specifically to detect and quantify HNF-1 activation. They contain a 96-well plate to which oligonucleotide containing a HNF-1 consensus sequence has been immobilized. HNF-1 dimers contained in nuclear cell extracts bind specifically to this oligonucleotide and are detected through use of an antibody directed against HNF-1. 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 HNF-1 Kits are available in two sizes:

product	format	catalog no.
TransAM HNF-1	1 x 96-well plate	46196
	5 x 96 well plates	46696

Active Motif also offers the TransAM HNF Family Kit for the study of HNF-1, -3α, -3β and -4α. See this and other Active Motif products related to HNF-1 in Appendix, Section B.

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

Flow Chart of Process



Introduction

HNF-1 Transcription Factor

Hepatocyte Nuclear Factor-1 (HNF-1) is a protein involved in liver development, metabolism and glucose homeostasis. HNF-1α and -1β (vHNF-1) are two related transcription factors of the homeodomain family.[2] Unlike other family members, HNF-1α and -1β have an extra 21 amino acid DNA binding segment in their homeobox, and a POU A-related domain that is involved in DNA binding.[3, 4] The N-terminus contains a dimerization domain allowing for homo- or heterodimerization. There are also three C-terminus activation domains that are rich in proline and glycine, proline and glutamine, and serine, respectively. HNF-1α is located on chromosome 12 and HNF-1β is found on chromosome 17.[4]

HNF-1 was originally discovered in the liver, where it was thought to be restricted. Later, it was also found to play important roles in the kidney, small intestine, thymus and pancreas. The HNF-1 consensus-binding site is the inverted palindrome GTTAATNATTAAC.[3] HNF-1 can bind to this sequence as a homodimer, but other factors, such as NF-1 or C/EBP, are required for efficient transcriptional activity.[3, 4] HNF-1 plays a crucial role in liver development during embryogenesis and metabolism homeostasis in the adult. It helps regulate such genes as glucose-cotransporter-2 in the kidney, phenylalanine hydroxylase (PAH) in the liver, and insulin, L-pyruvate kinase and aldolase B in pancreatic B cells.[4]

Mutations in HNF-1 have been implicated in the early onset of type II diabetes referred to as MODY (maturity onset diabetes of the young).[4] MODY is an inherited disease characterized by early onset of diabetes mellitus. MODY3 and 5 represent mutations in genes encoding HNF-1α and -1β, respectively. The most common form is MODY3, which accounts for about 60% of cases, while MODY5 is relatively rare. In all forms of MODY, insulin levels are effected by defective secretions of pancreatic B-cells.[4]

Transcription Factor Assays

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

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

2. The DNA-binding capacity of HNF 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 HNF binding. If HNF 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 cannot be applied to high-throughput screening. Gelshift assays also require special precautions and equipment for handling radioactivity.

3. Another method used to assay HNF activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing the HNF recognition site. The promoter can be artificial, made of several HNF elements or natural, like the Fibrinogen α 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 HNF-1

HNF-1 plays a key role in metabolism, liver development and glucose homeostasis, and therefore represents an excellent pharmacological target. However, pharmaceutical research in this field has been limited by the lack of convenient assays suitable for large numbers of samples.

To overcome this, Active Motif is introducing a high-throughput assay to quantify HNF-1 activation. The TransAM Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM HNF-1 Kits contain a 96-well plate on which has been immobilized an oligonucleotide that contains the HNF-1 consensus binding site (5´-GTTAATNATTAAC-3´). HNF-1 contained in nuclear extract binds specifically to this oligonucleotide. The primary antibody used in TransAM HNF-1 Kits recognizes an accessible epitope on HNF-1 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 HNF-1 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 HNF-1 activation using as little as 0.3 µg of nuclear extract from HepG2 cells. A comparable assay using EMSA requires 2.5 µg of nuclear extract and a 3-day autoradiography.

TransAM has many applications including the study of drug potency toward HNF-1, HNF-1 transcriptional activity regulation and protein structure/function studies of HNF-1.

Kit Performance and Benefits

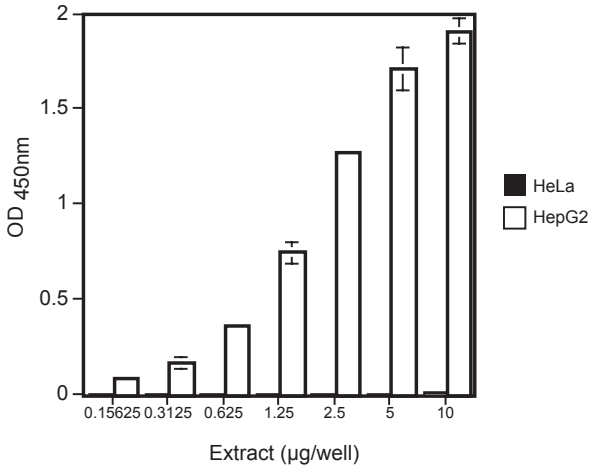
TransAM HNF-1 Kits are for research use only. Not for use in diagnostic procedures.

Detection limit: < 0.3 µg nuclear extract/well. TransAM HNF-1 is 5-fold more sensitive than EMSA.

Range of detection: TransAM provides quantitative results from 0.3 to 5 µg of nuclear extract/well (see graph below).

Cross-reactivity: The TransAM HNF-1 Kit detects mouse, rat and human HNF-1.

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



Monitoring HNF-1 activity with the TransAM HNF-1 Kit: Different amounts of nuclear extracts from untreated HepG2 and HeLa cells are tested for HNF-1 activity by using the TransAM HNF-1 Kit. This data is provided for demonstration only.

Kit Components and Storage

Except for the nuclear extract that must be kept at -80°C, 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
HNF-1 antibody	11 µl / 55 µl	4°C for 6 months
Anti-rabbit HRP-conjugated antibody	11 µl / 55 µl (0.2 µg/µl)	4°C for 6 months
Wild-type oligonucleotide AM15	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
Mutated oligonucleotide AM15	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
HepG2 nuclear extract	40 µl / 200 µl (2.5 µg/µl)	-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 AM2	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 HNF-1 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 cell 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 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 per ml of Binding Buffer AM2 (see the Quick Chart for Preparing Buffers in this section). After use, discard the 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, which makes it necessary to 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 10X Antibody Binding Buffer AM3 may form clumps, therefore you should homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use. Dilute the primary and secondary antibodies with the 1X Antibody Binding Buffer to 1:1000. 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).

HepG2 nuclear extract

The HepG2 nuclear extract is provided as a positive control for HNF-1 activation. Nuclear extract was made from unstimulated HepG2 cells. Sufficient extract is supplied for 40 reactions per plate. This extract is optimized to give a strong signal when used at 2.5 µg/well. We recommend aliquoting the extract in 5 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 HNF-1 binding in order to monitor the specificity of the assay. Used at 20 pmol/well, the oligonucleotide will prevent HNF-1 binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no effect on HNF-1 binding. Prepare the required amount of wild-type and/or mutated consensus oligonucleotide by adding 2 µl of appropriate oligonucleotide to 31.8 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 cell 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	0.02 µl	0.2 µl	1.2 µl	2.4 µl
	Protease Inhibitor Cocktail	0.23 µl	1.8 µl	10.8 µl	21.6 µl
	Lysis Buffer AM1	22.25 µl	178.0 µl	1.068 ml	2.136 ml
	TOTAL REQUIRED	**22.5 µl**	**180.0 µl**	**1.08 ml**	**2.16 ml**
Complete Binding Buffer	DTT	0.03 µl	0.3 µl	1.8 µl	3.6 µl
	Binding Buffer AM2	33.74 µl	269.7 µl	1.618 ml	3.232 ml
	TOTAL REQUIRED	**33.8 µl**	**270.0 µl**	**1.62 ml**	**3.24 ml**
Complete Binding Buffer with wild-type or mutated oligonucleotide	Wild-type or mutated oligo	2.0 µl	16.0 µl	96.0 µl	N/A
	Complete Binding Buffer	31.8 µl	254.0 µl	1.524 ml	N/A
	TOTAL REQUIRED	**33.8 µl**	**270.0 µl**	**1.62 ml**	**N/A**
1X Wash Buffer	Distilled water	2.025 ml	16.2 ml	97.2 ml	194.4 ml
	10X Wash Buffer AM2	225.0 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 Ab Binding Buffer AM3	22.5 µl	180 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.

HNF-1 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, 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 HNF-1 to its consensus sequence

1. Add 30 µl Complete Binding Buffer to each well to be used. If you wish to perform competitive binding experiments, add 30 µl Complete Binding Buffer that contains 20 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 20 µl of sample diluted in Complete Lysis Buffer per well. We recommend using 2-20 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 2.5 µg of the provided HepG2 nuclear extract diluted in 20 µl of Complete Lysis Buffer per well (1 µl of nuclear extract in 19 µl of Complete Lysis Buffer per well).

   Blank wells: Add only 20 µl Complete Lysis Buffer 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 HNF-1 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 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 2-20 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 the 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 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.

References

1. Wobser H., Dubmann H., Kogel D., Wang H., Reimertz C., Wollheim C., Byrne M.M. and Prehn J.H.M. (2002) *J. Biol. Chem.* 277(8): 6413-6421.
2. Wu G.D., Chen L., Forslund K. and Traber P.G. (1994) *J. Biol. Chem.* 269(25): 17080-17085.
3. Mendel D.B. and Crabtree G.R. (1991) *J. Biol. Chem.* 266(2): 677-680.
4. Ryffel G.U. (2001) *J. Mol. Endo.* 27: 11-29.

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 extract per well	Decrease amount of nuclear extract down to 1 µg/well
	Concentration of antibodies too high	Perform antibody titration to determine optimal working concentration. Start using 1:2000 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
	HNF-1 is poorly activated or inactivated in nuclear fractions	Perform a time course for HNF-1 activation in the studied cell line
	Extracts are not from rat, mouse or human origin	Perform study with a human, mouse or rat model

Section B. Related Products

Transcription Factor ELISAs	Unit	Catalog No.
TransAM™ C/EBP α/β	1 x 96 rxns	44196
	5 x 96 rxns	44696
TransAM™ HNF Family	2 x 96 rxns	46296
TransAM™ MyoD	1 x 96 rxns	47196
	5 x 96 rxns	47696
TransAM™ NFATc1	1 x 96 rxns	40296
	5 x 96 rxns	40796
TransAM™ NFκB Family	2 x 96 rxns	43296
TransAM™ NFκB p50	1 x 96 rxns	41096
	5 x 96 rxns	41596
TransAM™ NFκB p50 Chemi	1 x 96 rxns	41097
	5 x 96 rxns	41597
TransAM™ NFκB p65	1 x 96 rxns	40096
	5 x 96 rxns	40596
TransAM™ NFκB p65 Chemi	1 x 96 rxns	40097
	5 x 96 rxns	40597
TransAM™ NF-YA	1 x 96 rxns	40396
	5 x 96 rxns	40896
TransAM™ Sp1	1 x 96 rxns	41296
	5 x 96 rxns	41796
TransAM™ Sp1/Sp3	1 x 96 rxns	40496
	5 x 96 rxns	40996
TransAM™ STAT Family	2 x 96 rxns	42296
TransAM™ STAT3	1 x 96 rxns	45196
	5 x 96 rxns	45696

Supershift and Gelshift Assay Kits	Unit	Catalog No.
Nushift™ HNF-1α	17 rxns	37022
Nushift™ HNF-3α	17 rxns	37023
Nushift™ HNF-3γ	17 rxns	37051
Gelshift™ HNF-1	20 rxns	37332
Gelshift™ HNF-3	20 rxns	37311
Gelshift™ HNF-4	20 rxns	37312

Cell extracts	Unit	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410
Mitochondrial Fractionation Kit	100 rxns	40015
HepG2 nuclear extract	200 µg	36011

Antibodies	Application	Unit	Catalog No.
HNF-1α rabbit pAb	WB	100 µl	39030
HNF-1α rabbit pAb	SS	17 rxns	39321
HNF-3α rabbit pAb	SS	17 rxns	39322
HNF-3γ rabbit pAb	WB	100 µl	39031
HNF-3γ rabbit pAb	SS	17 rxns	39323
HNF-4 rabbit pAb	WB	100 µg	39076
C/EBPα rabbit pAb	WB, SS	100 µg	39306
C/EBPβ rabbit pAb	WB, SS	100 µl	39307
C/EBPδ rabbit pAb	WB	100 µl	39006
NF-1A rabbit pAb	WB	100 µl	39036
NF-1A rabbit pAb	WB, SS	100 µl	39329
NF-1B2 rabbit pAb	WB	100 µg	39091

mAb: monoclonal antibody; pAb: polyclonal antibody; WB: Western blot; SS: Supershift

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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