TransAM™

GATA-4

Transcription Factor Assay Kit

(version A1)

Catalog Nos. 46496 & 46996

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Overview

The GATA family consists of six members, GATA 1-6, that bind to the (A/T)GATA(A/G) nucleotide motif. GATA family members are grouped into two subfamilies based on structural and expression comparisons.[1] GATA-1, -2 and -3 are expressed predominantly in hematopoietic cells, and regulate differentiation and gene expression in T-lymphocytes, erythroids and megakaryocytes. GATA-4, -5 and -6 are found mainly in the heart and gut, and are involved in regulation of cardiogenesis and gut development.[1] Therefore, accurate monitoring of GATA activity 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.

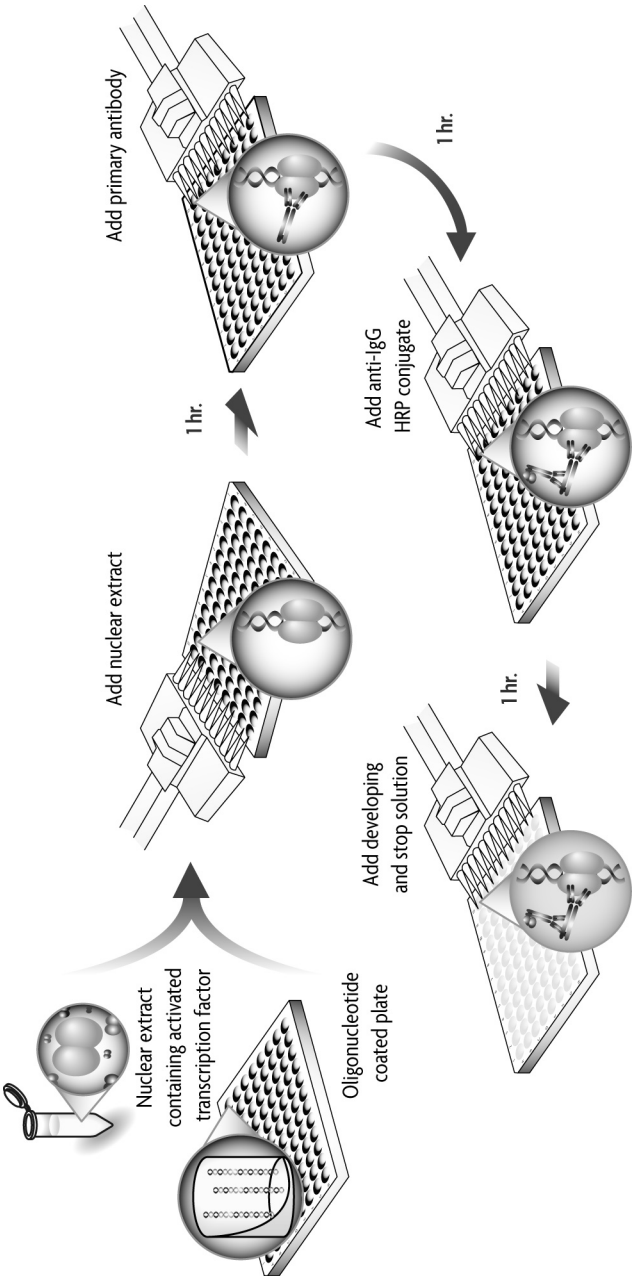
With its patented TransAM™ method*, Active Motif introduced the first ELISA-based kits to detect and quantify transcription factor activation. TransAM Kits combine a fast, user-friendly format with a sensitive, specific assay. TransAM GATA-4 Kits are designed specifically for the study of GATA-4 regulated pathways. They contain a 96-well plate to which oligonucleotide containing a GATA consensus binding site has been immobilized. By using an antibody that is directed against GATA-4, the GATA-4 complex bound to the oligonucleotide is detected. 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 high-throughput screening applications. TransAM GATA-4 Kits are available in two sizes:

product	format	catalog no.
TransAM GATA-4	1 x 96-well plate	46496
	5 x 96 well plates	46996

Active Motif also offers the TransAM GATA Family Kit for the study of GATA-1, -2 and -3. See this and other Active Motif products related to the GATA-4 transcription factor in Appendix, Section B.

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

Flow Chart of Process



GATA Transcription Factor

The GATA family of transcription factors consists of 6 members: GATA 1-6. The family is defined by the presence of 1 or 2 zinc fingers containing four cysteine residues with the motif $CX_2CX_{12}CX_2C$ that coordinate a single zinc ion.[2] In mammals, the N-finger is important for interactions with cofactors such as Friend of GATA (FOG), while the C-finger binds to (A/T)GATA(A/G) motifs.[2] Genes encoding GATA factors are transcriptionally regulated in a tissue-restricted manner.[3] They include platelet factor 4 (PF4), glycoprotein (GP) IIB, α-myosin heavy chain, thyroid transcription factor-1 (TTF-1) and H+/K+-ATPase.[4] The members of the GATA family have been grouped into two subfamilies: GATA-1, -2 and -3 are expressed mainly in hematopoietic cells, and GATA-4, -5 and -6 are found predominantly in the heart and gut.

GATA-1, the founding member of the family, is essential for normal erythropoiesis and megakaryocyte differentiation.[5] It is expressed in erythroid cells, megakaryocytes, mast cells, eosinophils and testis.[3] Thought to be a negative regulator of cell proliferation in early megakaryocyte progenitors[4], GATA-1 has also been implicated in regulation of terminal differentiation markers in various myeloid cells.[3] Mutations in GATA-1 are associated with familial blood disorders.[2] GATA-2 is expressed in vascular endothelial cells and embryonic brain and liver.[3] It is thought to play a role in the regulation of endothelial gene expression[5], and is also found in megakaryocytes and mast cells with GATA-1. GATA-3 is found mainly in cells of T-cell lineage, and in low levels in mast cells.[5] It is detected in the most immature subset of fetal day 12 thymocytes, and is essential for T-cell development.[6]

GATA-4, -5 and -6 regulate gene expression in the heart, gut epithelium, liver, lung and gonad.[1] Target genes of GATA-4, -5 and -6 include A- and B-type natriuretic peptides, cardiac actin and α-myosin heavy chain. GATA-4 is the transcriptional activator of several cardiac muscle-specific genes and a key regulator of the cardiomyocyte gene program. It can be found in the adult heart, ovary, testis, lung, liver and small intestine.[1] During development, GATA-4 is expressed in the heart, gut, testis, ovary, liver, visceral endoderm and parietal endoderm.[1] GATA-5 is expressed in the allantois, heart, lung bud, urogenital ridge, bladder and gut epithelium during development, and can be found in the adult small intestine, stomach, bladder and lungs.[1] GATA-6 is thought to regulate the proliferation of cardiac progenitor cells. During development, it is expressed in the primitive streak, allantois, visceral endoderm, heart, lung buds, urigenital ridge, vascular smooth muscle cells and epithelial layer of the stomach, small intestine and large intestine. In adults, GATA-6 is found in the heart, aorta, stomach, small intestine and bladder.[1]

Transcription Factor Assays

To date, three methods are widely used to measure GATA-4 activation, either directly or indirectly:

1. GATA-4 expression can be measured by Western blot, using antibodies raised against GATA-4 subunits. 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 GATA-4 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 GATA-4 binding. If GATA-4 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 GATA-4 activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing the GATA-4 consensus sequence. This promoter can be artificial, made of several GATA cis-elements and a TATA box, or natural, like the mouse α-globin GATA site. Limitations of this procedure are: (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 GATA-4

GATA-4 is implicated as an important regulator of gene expression in the heart, liver, gonad, gut epithelium and lung, thus representing 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 GATA-4 activation.[7] The TransAM Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM GATA-4 Kits contain a 96-well plate on which has been immobilized oligonucleotide containing the GATA consensus binding site 5´-AGATAA-3´. GATA-4 contained in nuclear extract binds specifically to this oligonucleotide. The primary antibody used in TransAM GATA-4 Kits recognize an epitope on GATA-4 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 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 GATA-4 and has been shown to be 5-fold more sensitive and 20-fold faster than the gel-retardation tech-

nique. With the 3.5-hour TransAM procedure, we could detect GATA-4 activation using as little as 1 µg of nuclear extract. A comparable assay using EMSA requires 5 µg of nuclear extract and a 3-day autoradiography.

TransAM has many applications including the study of drug potency, GATA-4 transcriptional activity regulation and protein structure/function studies of GATA-4 signaling pathways.

Kit Performance and Benefits

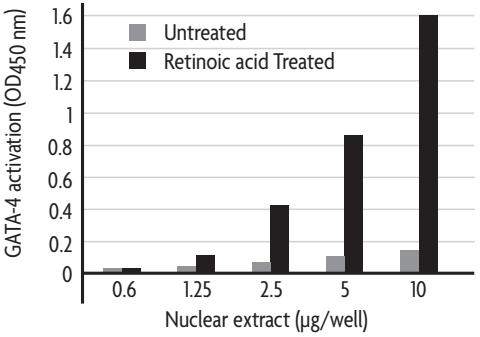
The TransAM GATA-4 Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: < 1.0 µg nuclear extract/well. The TransAM GATA-4 Kit is up to 5-fold more sensitive than EMSA.

Range of detection: TransAM provides quantitative results from 1 to 10 µg of nuclear extract/well.

Cross-reactivity: TransAM GATA-4 detects GATA-4 from human origin. Reactivity with other species has not been determined.

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



Monitoring GATA-4 activity using the TransAM GATA-4 Kit. Different amounts of nuclear extracts from untreated and Retinoic acid treated F9 cells are tested for GATA-4 activation by using the TransAM GATA-4 Kit. This data is provided for demonstration purposes 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
GATA-4 antibody	11 µl / 55 µl	-20°C for 6 months
Anti-rabbit HRP-conjugated IgG	11 µl / 55 µl (0.25 µg/µl)	4°C for 6 months
Wild-type oligonucleotide AM12	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
Mutated oligonucleotide AM12	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
F9 nuclear extract (Retinoic acid treated)	40 µl / 200 µl (2.5 µg/µl)	-80°C for 6 months
Herring sperm DNA	100 µl / 500 µl (1 µg/µl)	-20°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 GATA 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 1 µl of 1 M DTT and 10 µl of Herring sperm DNA 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 vortexing for 2 minutes 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 AM3 required, dilute 1 ml 10X Antibody Binding Buffer 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 both primary and 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 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

F9 cells treated with Retinoic acid are provided as a positive control for GATA-4. Sufficient extract is supplied for 20 reactions. 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 -80°C. Avoid multiple freeze/thaw cycles of the extract. Various nuclear 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 GATA-4 binding in order to monitor the specificity of the assay. Used at 20 pmol/well, the oligonucleotide will prevent GATA-4 binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no effect on GATA-4 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 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	0.02 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
	Herring sperm DNA	0.34 µl	2.7 µl	16.2 µl	32.4 µl
	Binding Buffer AM2	33.4 µl	267.0 µl	1.602 ml	3.2 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 µl	16 µl	96 µl	N/A
	Complete Binding Buffer	31.8 µl	254 µl	1.524 ml	N/A
	TOTAL REQUIRED	**33.8 µl**	**270 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 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.

GATA-4 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 GATA-4 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 5 µg of the provided nuclear extract diluted in 20 µl of Complete Lysis Buffer per well (2 µl of nuclear extract in 18 µl of Complete Lysis Buffer per well).

   Blank wells: Add 20 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 GATA-4 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 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 room-temperature Developing Solution to all wells being used.
2. Incubate 2-10 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 confluent cell layer of 75 cm^2 (100 mm dish). The yield is approximately 0.5 mg of nuclear proteins for 10^7 cells.

1. Wash cells with 10 ml of ice-cold PBS/PIB.
2. Add 10 ml of ice-cold PBS/PIB and scrape the cells off the dish with a cell lifter. Transfer the 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 of 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 mix by gentle pipetting.
6. Centrifuge the homogenate for 30 seconds at 4°C in a microcentrifuge.
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 of PIB to 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.

References

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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 extract per well	Decrease amount of nuclear extract down to 1-2 µg/well
	Concentration of antibody 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
	GATA-4 is poorly activated or inactivated	Perform a time course for GATA-4 activation in the studied cell line
	Nuclear extracts are not from correct species	Refer to cross-reactivity information on page 5

Section B. Related Products

Kits	Unit	Catalog No.
TransAM™ C/EBP α/β	1 x 96 rxns	44196
	5 x 96 rxns	44696
TransAM™ GATA Family	2 x 96 rxns	48296
TransAM™ HNF Family	2 x 96 rxns	46296
TransAM™ HNF-1	1 x 96 rxns	46196
	5 x 96 rxns	46696
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

DNA Repair Kits	Unit	Catalog No.
GTBP DNA Repair Kit	1 x 96 rxns	51096
	5 x 96 rxns	51596
Ku70/86 DNA Repair Kit	1 x 96 rxns	51196
	5 x 96 rxns	51696

Supershift and Gelshift Assay Kits	Unit	Catalog No.
Nushift™ GATA-1	17 rxns	37039
Gelshift™ GATA-1	20 rxns	37324

Cell extracts	Unit	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410
Mitochondrial Fractionation Kit	100 rxns	40015
F9 nuclear extract	200 µg	36007
F9 nuclear extract (Retinoic acid treated)	200 µg	36113

Antibodies	Application	Unit	Catalog No.
GATA-1 rabbit pAb	WB	100 µl	39025
GATA-1 rabbit pAb	SS	17 rxns	39319
GATA-3 rabbit pAb	WB	100 µl	39026
TTF-1 mouse mAb	WB	100 µl	39525

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.

Active Motif North America
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Telephone: 760 431 1263
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