TransAM™

AML-1/Runx1
Transcription Factor Assay Kits

(version B)

Catalog Nos. 47396 & 47896

Active Motif North America
1914 Palomar Oaks Way, Suite 150
Carlsbad, California 92008, USA
Toll free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351

Active Motif Europe
1104 Avenue Franklin Roosevelt
B-1330 Rixensart, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050

Active Motif Japan
Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733

TABLE OF CONTENTS **Page**

Overview

AML-1 (Runx1, CBFA2, PEBP2αB1) is a transcription factor that is essential for definitive hematopoiesis and is one of the most frequently mutated genes in human leukemia[1]. Because of the importance of AML-1 in normal development, accurate monitoring of AML-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.

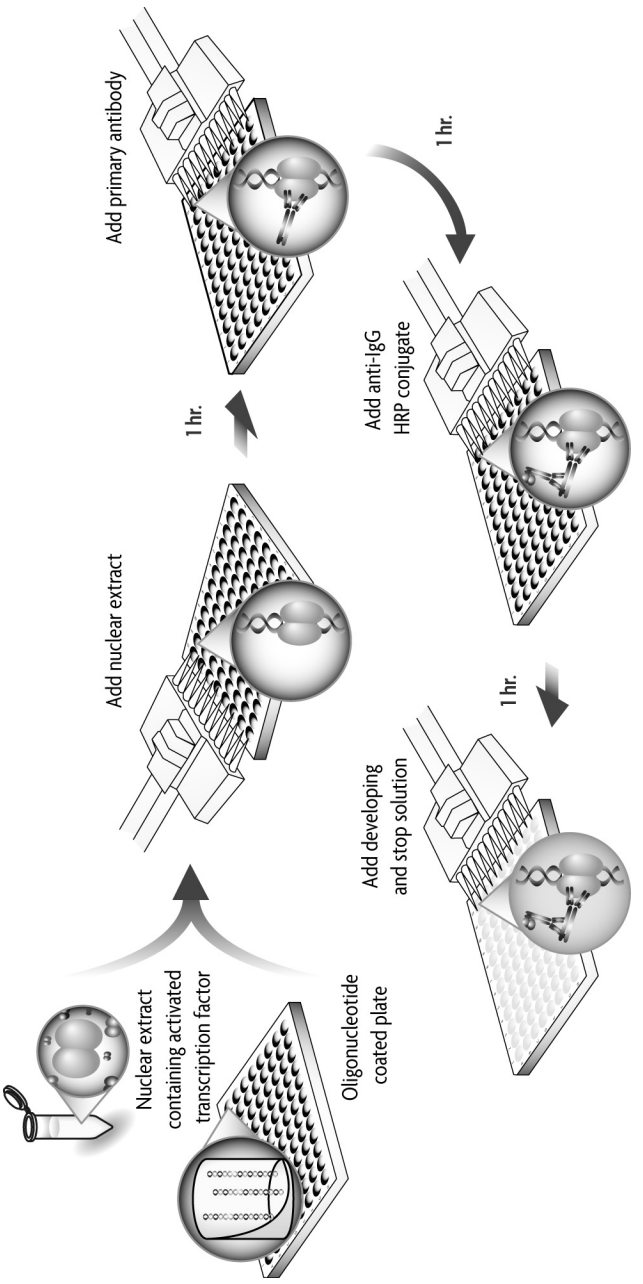
With its patented TransAM™ method*, Active Motif introduced the first ELISA-based kits to detect and quantify transcription factor activation. TransAM Kits combine a fast, user-friendly format with a sensitive, specific assay. TransAM AML-1/Runx1 Kits are designed specifically for the study of AML-1/Runx1. They contain a 96-well plate to which oligonucleotide containing an AML-1 consensus binding site has been immobilized. The activated AML-1 contained in nuclear extract specifically binds to this oligonucleotide. By using an antibody that is directed against AML-1, the 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 AML-1/Runx1 Kits are available in two sizes:

product	format	catalog no.
TransAM AML-1/Runx1	1 x 96-well plate	47396
	5 x 96 well plates	47896

See Active Motif products related to the AML/Runx family in Appendix, Section B.

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

Flow Chart of Process



AML/Runx Transcription Factor

AML-1 (Runx1, CBFA2, PEBP2αB1) is the evolutionarily conserved DNA-binding subunit of a transcription complex known as core-binding factor (CBF)[1]. AML/Runx factors are essential for blood, skeletal and gastric development[2] and are composed of heterodimeric α and β subunits[3]. In mammals, the α subunits are encoded by three genes, Runx1, 2 and 3, while the β subunit (PEBP2β, CBFβ) is ubiquitously expressed[3]. AML-1 /Runx1 is essential for hemopoietic development while AML-3/Runx2 controls osteoblast differentiation and the development of hypertrophic cartilage. All α subunits share a highly conserved DNA binding domain: runt[3].

AML/Runx factors interact with numerous transcription factors (AP-1, C/EBP, Ets-1) and co-factors (p300, ALY, TLE) to regulate tissue-specific gene expression[4]. AML/Runx proteins consist of the Runt domain, the transcription activation domain and the inhibition domain[5]. The Runt domain is responsible for DNA binding and heterodimerization with PEBP2β[5]. This subunit enhances DNA binding and stabilizes the proteins against proteolytic degradation.

AML-1 activates the transcription of numerous genes including genes encoding cytokines, T cell receptors and myeloid-specific genes[6]. AML-1 can also associate with the Groucho and mSin3 co-repressors to repress transcription[6]. Mutation of AML-1 is associated with both acute myeloid leukemia and childhood B-cell acute lymphoblastic leukemia[6]. Since the wild type form of AML-1 is transforming when expressed in fibroblasts, it is also considered an oncogene. Therefore, AML-1 is unusual in that both wild type and mutated forms are associated with cancer.

Transcription Factor Assays

To date, three methods are widely used to measure AML-1/Runx1 activation, either directly or indirectly:

1. AML-1 expression can be measured by Western blot, using antibodies raised against AML-1. 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 AML-1 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 AML-1 binding. If AML-1 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 AML-1 activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing the AML-1

consensus sequence. This promoter can be artificial, made of several AML-1 cis-elements and a TATA box, or natural. 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 AML-1/Runx1

AML-1 is essential for definitive hematopoiesis, which makes it an excellent pharmacological target. However, pharmaceutical research in this field has been hampered by the lack of convenient assays suitable for processing large numbers of samples.

To overcome this, Active Motif is offering a high-throughput assay to quantify AML-1 activation. The TransAM Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM AML-1/Runx1 Kits contain a 96-well plate on which has been immobilized oligonucleotide containing the AML-1 consensus binding site (5´-AACCACA-3´). The active form of AML-1 contained in nuclear extract specifically binds to this oligonucleotide. The primary antibodies used to detect AML-1 recognize an epitope on AML-1 that is accessible only when AML-1 is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides a sensitive colorimetric readout that is easily quantified by spectrophotometry. Once the cellular extract is prepared, this assay is completed in less than 3.5 hours. As this assay is performed in a 96-well plate, a large number of samples can be handled simultaneously, allowing for high-throughput automation. This assay is specific for AML-1 activation and has been shown to be 10-fold more sensitive and 20-fold faster than the gel retardation technique. With the 3.5-hour procedure of TransAM, we could detect AML-1 activation with 0.5 µg of nuclear extract. A comparable assay using EMSA required 5 µg of nuclear extract and a 5-day autoradiography.

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

Kit Performance and Benefits

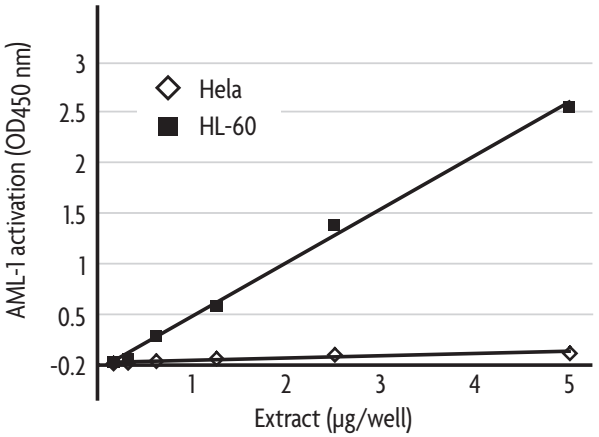
The TransAM AML-1/Runx1 Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: < 0.6 µg nuclear extract/well. TransAM is 10-fold more sensitive than EMSA.

Range of detection: TransAM provides quantitative results from 0.6 to 10 µg of nuclear extract/well.

Cross-reactivity: TransAM AML-1/Runx1 specifically detects bound AML-1/Runx1 in human samples..

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



Monitoring AML-1/Runx1 activation with the TransAM AML-1/Runx1 Kit: Different amounts of nuclear extracts from unstimulated HeLa and HL-60 cells are tested for AML-1/Runx1 activation by using the TransAM AML-1/Runx1 Kit. These curves are 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
AML-1/Runx1 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 AM24	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
Mutated oligonucleotide AM24	100 µl / 500 µl (10 pmol/µl)	-20°C for 6 months
HL-60 nuclear extract	40 µl / 200 µl (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
Herring sperm DNA	100 µl / 500 µl (1 µg/µl)	-20°C for 6 months
Lysis Buffer AM4	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 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 AM4 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 of Protease Inhibitor Cocktail per ml of Lysis Buffer AM4 (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 1 µg/µl 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 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 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.

Diluted Primary Antibody

The primary AML-1 antibody is used to detect the active AML-1 protein bound to the plate. The supplied antibody will be diluted 1:4000 in 1X Antibody Binding Buffer. This dilution should be made by performing a 1:40 dilution followed by a 1:100 dilution (see the Quick Chart for Preparing Buffers in this section).

Diluted HRP-conjugated Secondary Antibody

HRP-conjugated anti-rabbit IgG is used as the secondary antibody to detect bound primary antibody. The supplied antibody will be diluted 1:1000 in 1X Antibody Binding Buffer (see the Quick Chart for Preparing Buffers in this section).

Developing Solution

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

Stop Solution

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

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

Nuclear extract

The HL-60 nuclear extract is provided as a positive control for AML-1 activation. Sufficient extract is supplied for 40 reactions per plate. This extract is optimized to give a strong signal when used at 5 µg/well. We recommend aliquoting the extract in 5 µl fractions and storing at -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 AML-1 binding in order to monitor the specificity of the assay. Used at 10 pmol/well, the oligonucleotide will prevent AML-1 binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no effect on AML-1 binding. Prepare the required amount of wild-type and/or mutated consensus oligonucleotide by adding 1 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 AM4	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	0.27 µl	1.6 µl	3.25 µl
	Herring sperm DNA	0.34 µl	2.7 µl	16.2 µl	32.4 µl
	Binding Buffer AM2	33.4 µl	267 µl	1.6 ml	3.2 ml
	TOTAL REQUIRED	**33.8 µl**	**270 µl**	**1.62 ml**	**3.24 ml**
Complete Binding Buffer with wild-type or mutated oligonucleotide	Wild-type or mutated oligo	1 µl	9 µl	54 µl	N/A
	Complete Binding Buffer	31.8 µl	261 µl	1.566 ml	N/A
	TOTAL REQUIRED	**32.8 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	252 µl	1.8 ml	9.9 ml	19.8 ml
	10X Ab Binding Buffer AM3	28 µl	200 µl	1.1 ml	2.2 ml
	TOTAL REQUIRED	**280 µl**	**2.0 ml**	**11 ml**	**22 ml**
Primary Antibody Pre-Dilution (1/40)	AML-1 antibody	1 µl	1 µl	2 µl	3 µl
	1X Ab Binding Buffer	39 µl	39 µl	78 µl	117 µl
	TOTAL REQUIRED	**40 µl**	**40 µl**	**80 µl**	**120 µl**
Primary Antibody 1/100 Dilution	Pre-diluted AML-1 antibody	1.1 µl	9 µl	52 µl	104 µl
	1X Ab Binding Buffer	110 µl	891 µl	5.148 ml	10.296 ml
	TOTAL REQUIRED	**110.11 µl**	**900 µl**	**5.2 ml**	**10.4 ml**
Secondary Antibody	HRP conjugated antibody	0.11 µl	0.9 µl	5.2 µl	10.4 µl
	1X Ab Binding Buffer	110 µl	900 µl	5.2 ml	10.4 ml
	TOTAL REQUIRED	**110.11 µl**	**900.9 µl**	**5.2 ml**	**10.4 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.

** The Quick Chart includes an excess of components to perform the assay.

AML-1/Runx1 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 AML-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 10 pmol (1 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 is provided on page 11.

   Positive control wells: Add 5 µg of the provided nuclear extract diluted in 20 µl of Complete Lysis Buffer per well (1 µl of extract in 19 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 Washing 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 antibody (1:4000 dilution in 1X Antibody Binding Buffer, see Quick Chart for preparing Buffers on page 9 for details on performing dilution) 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 Washing Buffer (as described in Step 1, No. 4).

Step 3: Binding of secondary antibody

1. Add 100 µl diluted HRP 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 Washing 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 the 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 cell 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

1. Kamaleldin E. *et al* (2003) *Blood* 101(11): 4333-4341.
2. Westendorf J.J. *et al* (2002) *Molecular & Cellular Biology* 22: 7982-7992.
3. Vaillant F. *et al* (2002) *J. Immunology* 169: 2866-2874.
4. Kahler R.A. *et al* (2003) *J. Biol. Chem.* 278(14): 11937-11944.
5. Wee H.J. *et al* (2002) *EMBO* 3(10): 967-974.
6. Strom D..K. *et al* (2000) *J. Biol. Chem.* 275(5): 3438-3445.

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 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 50 µg/well
	AML-1 is poorly activated or inactivated	Perform a time course for AML-1 activation in the studied cell line
	Extracts are not from correct species	Refer to cross-reactivity information on pg. 5

Section B. Related Products

TransAM™ Kits	Format	Catalog No.
TransAM™ AML-3/Runx2	1 x 96 rxns	44496
	5 x 96 rxns	44996
TransAM™ AP-1 Family	2 x 96 rxns	44296
TransAM™ AP-1 c-Fos	1 x 96 rxns	44096
	5 x 96 rxns	44596
TransAM™ AP-1 FosB	1 x 96 rxns	45096
	5 x 96 rxns	45596
TransAM™ AP-1 c-Jun	1 x 96 rxns	46096
	5 x 96 rxns	46596
TransAM™ AP-1 JunD	1 x 96 rxns	43496
	5 x 96 rxns	43996
TransAM™ ATF-2	1 x 96 rxns	42396
	5 x 96 rxns	42896
TransAM™ c-Myc	1 x 96 rxns	43396
	5 x 96 rxns	43896
TransAM™ C/EBP α/β	1 x 96 rxns	44196
	5 x 96 rxns	44696
TransAM™ CREB	1 x 96 rxns	42096
	5 x 96 rxns	42596
TransAM™ pCREB	1 x 96 rxns	43096
	5 x 96 rxns	43596
TransAM™ Elk-1	1 x 96 rxns	44396
	5 x 96 rxns	44896
TransAM™ HIF-1	1 x 96 rxns	47096
	5 x 96 rxns	47596
TransAM™ HNF Family	2 x 96 rxns	46296
TransAM™ IRF Family	2 x 96 rxns	45296
TransAM™ MAPK Family	2 x 96 rxns	47296
TransAM™ MEF2	1 x 96 rxns	43196
	5 x 96 rxns	43696
TransAM™ NFκB Family	2 x 96 rxns	43296
TransAM™ NFκB p50 Chemi	1 x 96 rxns	41097
	5 x 96 rxns	41597
TransAM™ NFκB p65 Chemi	1 x 96 rxns	40097
	5 x 96 rxns	40597
TransAM™ STAT Family	2 x 96 rxns	42296
TransAM™ STAT3	1 x 96 rxns	45196
	5 x 96 rxns	45696

Nushift™ & Gelshift™ Kits	Format	Catalog No.
Nushift™ AML/Runx Family	17 rxns	37000
Nushift™ AML-1/Runx1	17 rxns	37001
Nushift™ AML-2/Runx3	17 rxns	37046
Nushift™ AML-3/Runx2	17 rxns	37002
Gelshift™ AML-1/Runx1	20 rxns	37300
Gelshift™ AML-2/Runx3	20 rxns	37328
Gelshift™ AML-3/Runx2	20 rxns	37329

Cell-based ELISAs	Format	Catalog No.
FACE™ AKT	1 x 96 rxns	48120
	5 x 96 rxns	48620
FACE™ AKT Chemi	1 x 96 rxns	48220
	5 x 96 rxns	48720
FACE™ ATF-2	1 x 96 rxns	48115
	5 x 96 rxns	48615
FACE™ ATF-2 Chemi	1 x 96 rxns	48215
	5 x 96 rxns	48715
FACE™ ERK1/2	1 x 96 rxns	48140
	5 x 96 rxns	48640
FACE™ ERK1/2 Chemi	1 x 96 rxns	48240
	5 x 96 rxns	48740
FACE™ GSK3β	1 x 96 rxns	48170
	5 x 96 rxns	48670
FACE™ GSK3β Chemi	1 x 96 rxns	48270
	5 x 96 rxns	48770
FACE™ JNK	1 x 96 rxns	48110
	5 x 96 rxns	48610
FACE™ JNK Chemi	1 x 96 rxns	48210
	5 x 96 rxns	48710
FACE™ MEK1/2	1 x 96 rxns	48180
	5 x 96 rxns	48680
FACE™ MEK1/2 Chemi	1 x 96 rxns	48280
	5 x 96 rxns	48780
FACE™ p38	1 x 96 rxns	48100
	5 x 96 rxns	48600
FACE™ p38 Chemi	1 x 96 rxns	48200
	5 x 96 rxns	48700

Cell extracts	Format	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40510
Mitochondrial Fractionation Kit	100 rxns	40015
HeLa nuclear extract	200 µg	36010
HL-60 nuclear extract	200 µg	36072

Antibodies	Application	Format	Catalog No.
AML-1/Runx1 mAb	WB	100 µl	39500
AML-1/Runx1 pAb	WB, SS	100 µl	39000
AML-2/Runx3 Nushift™	WB, SS	200 µl	39301
AML-3/Runx2 Nushift™	WB, SS	200 µl	39302

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
1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008
USA
Toll Free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351
E-mail: tech_service@activemotif.com

Active Motif Europe
104 Avenue Franklin Roosevelt
B-1330 Rixensart, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan
Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733
E-mail: japantech@activemotif.com

Visit Active Motif on the worldwide web at **http://www.activemotif.com**

At this site:

- Read about who we are, where we are, and what we do
- Review data supporting our products and the latest updates
- Enter your name into our mailing list to receive our catalog, *MotifVations* newsletter and notification of our upcoming products
- Share your ideas and results with us
- View our job opportunities

Don't forget to bookmark our site for easy reference!