TransAM® Nrf2

(version A4)

Catalog Nos. 50296 & 50796

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Revision History

Revision	Date	Description of Change
A4	Feb 2020	Nrf2 antibody now stored at -20°C

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Overview

NF-E2-related factor, Nrf2, is a critical transcription factor in oxidative stress signaling. Nrf2 is a basic leucine zipper transcription factor that binds to the antioxidant responsive element (ARE) and may serve as a master regulator in cellular defense pathways in protecting a wide variety of tissues from various toxic exposure.

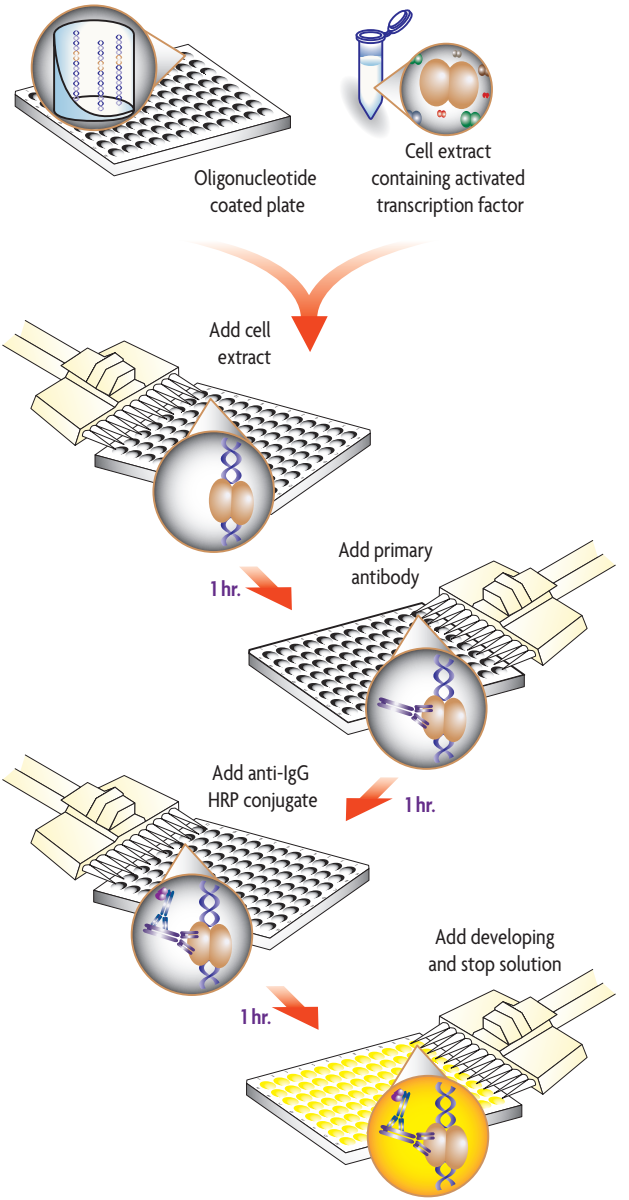
TransAM® Nrf2 is a simple solution for studying Nrf2 activation in human, mouse and rat model systems. The Kit is a 96-well plate based assay measuring DNA binding activity of Nrf2 using colorimetric detection on a standard plate reader. It works with nuclear extracts from tissue or cell samples and is able to detect activated Nrf2 in as little as 0.15 micrograms of nuclear extract.

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 Nrf2 Kits are designed specifically to detect and quantify Nrf2 activation. Kits contain a 96-well plate to which oligonucleotide containing an ARE has been immobilized. Nrf2 contained in nuclear extract then binds specifically to this oligonucleotide and is detected through use of an antibody directed against Nrf2. 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® Nrf2	1 x 96 rxns	50296
TransAM® Nrf2	5 x 96 rxns	50796

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

Flow Chart of Process



Introduction

Nrf2 Transcription Factor

Nrf2 (NF-E2 related factor, NFE2L2, from nuclear factor erythroid-derived 2-like 2) is a basic leucine zipper (bZIP) transcription factor. Nrf2 binds to the antioxidant response element (ARE) and positively regulates the expression of detoxifying enzyme genes (such as NAD(P)H:quinone oxidoreductase1, NQO1) in response to antioxidants and xenobiotics. Higher levels of NQO1 gene expression has been shown in liver, lung, colon, and breast tumors[1].

A cytosolic inhibitor of Nrf2, Keap1/INrf2, retains Nrf2 in the cytoplasm under normal conditions where the interaction of Nrf2 with INrf2 targets Nrf2 for ubiquitination and proteasomal degradation. However, after oxidative stress, Nrf2 is released from INrf2, translocates to the nucleus, and results in the activation of ARE-mediated gene expression. Nrf2 is also synthesized *de novo* after exposure to stress. In addition, Nrf2 controls its own degradation by regulating expression and induction of INrf2[2]. It has been shown that nuclear export and degradation pathways are activated by around two hours after treatment with tert-butylhydroquinone (t-BHQ)[3].

Nrf2 activation and degradation are important sensing mechanisms in the cellular response for oxidative and electrophilic stressors[4]. The TransAM Nrf2 Kit provides a simple solution for studying Nrf2 activation levels in tissue or cell extracts.

Transcription Factor Assays

To date, three methods are widely used to measure Nrf2 activation, either directly or indirectly:

1. Nrf2 activation can be determined by Western blot by using antibodies specific for Nrf2 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 Nrf2 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 Nrf2 binding. If Nrf2 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 Nrf2 activation is based on reporter genes, typically luciferase or β-galactosidase, placed under the control of a promoter containing the ARE consensus 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. 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 Nrf2

The TransAM Nrf2 Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM Nrf2 Kits contain a 96-well plate on which has been immobilized oligonucleotide containing the ARE consensus binding site (5´-GTCACAGTGACTCAG-CAGAATCTG-3´). The active form of Nrf2 contained in nuclear extract specifically binds to this oligonucleotide. The primary antibody used to detect Nrf2 recognizes an epitope on Nrf2 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 Nrf2 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 Nrf2 activation using as little as 0.6 µg of nuclear extract.

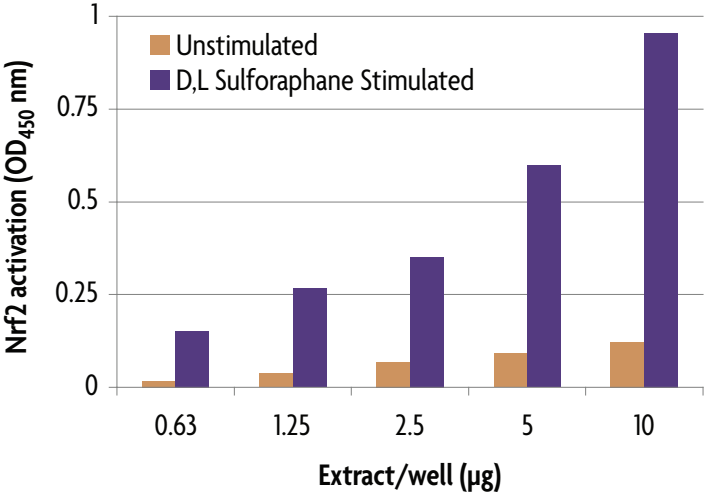
Kit Performance and Benefits

Detection limit: > 0.6 µg nuclear extract/well.

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

Cross-reactivity: TransAM Nrf2 specifically detects Nrf2 from human, mouse and rat origin.

Assay time: 3.5 hours.



Monitoring Nrf2 activation with the TransAM Nrf2 Kit:

0.625 to 10 µg of D,L Sulforaphane treated HepG2 nuclear extract (purple bars) and untreated HepG2 nuclear extract (copper bars) were assayed per well. Data shown are the results from wells assayed in duplicate.

Kit Components and Storage

TransAM Nrf2 Kits are for research use only. Not for use in diagnostic procedures. Except for the positive control 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. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity 1 plate / 5 plates	Storage
Nrf2 antibody	10 µl / 25 µl	-20°C
anti-rabbit HRP-conjugated antibody	10 µl / 50 µl	4°C
Wild-type oligonucleotide AM29	100 µl / 500 µl (10 pmol/µl)	-20°C
Mutated oligonucleotide AM29	100 µl / 500 µl (10 pmol/µl)	-20°C
Positive control extract	20 µl / 50 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
Herring Sperm DNA	100 µl / 500 µl (1 µg/µl)	-20°C
Lysis Buffer AM1	10 ml / 50 ml	4°C
Binding Buffer AM1	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 Nrf2 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 transcription factors 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 1 µg/µl Herring Sperm DNA per ml of Binding Buffer AM1 (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 Nrf2 and HRP-conjugated 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.

Positive control extract (2.5 µg/µl)

The positive control nuclear extract is provided as a control for Nrf2 activation. Sufficient extract is supplied for 20 reactions if using 5 µg per well. This extract is optimized to give a strong signal when used at 0.6 to 10 µg/well. We recommend aliquoting the extract in 5 µl fractions and storing at -80°C. 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 Nrf2 binding in order to monitor the specificity of the assay. Used at 20 pmol/well, the oligonucleotide will prevent Nrf2 binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no inhibitory effect on Nrf2 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
	Herring Sperm DNA	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 Nrf2 wt or mut oligont	wt or mut oligont	2.0 µl	18.0 µl	108 µl	N/A
	Complete Binding Buffer	43.0 µl	342.0 µl	2.052 ml	N/A
	Total Required	**45.0 µl**	**360.0 µl**	**2.16 ml**	**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.

Nrf2 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 Nrf2 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 5-20 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 positive control extract diluted in 10 µl of Complete Lysis Buffer per well (2 µl of control extract in 8 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 Nrf2 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.

References

1. Pi, J., *et al.* (2007) *Free Radic Biol Med* 42(12): 1797-1806.
2. Lee, O-H., *et al.* (2007) *J Biol Chem* 282(50): 36412-36420.
3. Theodore, M., *et al.* (2008) *J Biol Chem* 283(14): 8984-8994
4. Kobayashi, A., *et al.* (2006) *Mol Cell Biol* 26(1): 221-229.

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 1:1000 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
	Nrf2 is poorly activated or inactivated in nuclear fractions	Perform a time course for Nrf2 activation in the studied cell line
	Nuclear extracts are not from correct species	

Section C. Related Products

Transcription Factor ELISAs	Format	Catalog No.
TransAM® AML-1/Runx1	1 x 96-well plate	47396
TransAM® AML-3/Runx2	1 x 96-well plate	44496
TransAM® AP-1 Family	2 x 96-well plates	44296
TransAM® AP-1 c-Fos	1 x 96-well plate	44096
TransAM® AP-1 c-Jun	1 x 96-well plate	46096
TransAM® AP-1 FosB	1 x 96-well plate	45096
TransAM® AP-1 JunD	1 x 96-well plate	43496
TransAM® ATF-2	1 x 96-well plate	42396
TransAM® c-Myc	1 x 96-well plate	43396
TransAM® C/EBP α/β	1 x 96-well plate	44196
TransAM® CREB	1 x 96-well plate	42096
TransAM® pCREB	1 x 96-well plate	43096
TransAM® Elk-1	1 x 96-well plate	44396
TransAM® ER	1 x 96-well plate	41396
TransAM® FKHR (FOXO1/4)	1 x 96-well plate	46396
TransAM® GATA Family	2 x 96-well plates	48296
TransAM® GATA-4	1 x 96-well plate	46496
TransAM® GR	1 x 96-well plate	45496
TransAM® HIF-1	1 x 96-well plate	47096
TransAM® HNF Family	2 x 96-well plates	46296
TransAM® HNF-1	1 x 96-well plate	46196
TransAM® IRF-3 (Human)	1 x 96-well plate	48396
TransAM® IRF-3 (Mouse)	1 x 96-well plate	48496
TransAM® IRF-7	1 x 96-well plate	50196
TransAM® MAPK Family	2 x 96-well plates	47296
TransAM® MEF2	1 x 96-well plate	43196
TransAM® MyoD	1 x 96-well plate	47196
TransAM® NF-YA	1 x 96-well plate	40396
TransAM® NFATc1	1 x 96-well plate	40296
TransAM® NFκB Family	2 x 96-well plates	43296
TransAM® Flexi NFκB Family	2 x 96-well plates	43298
TransAM® NFκB p50	1 x 96-well plate	41096
TransAM® NFκB p50 Chemi	1 x 96-well plate	41097
TransAM® Flexi NFκB p50	1 x 96-well plate	41098
TransAM® NFκB p52	1 x 96-well plate	48196
TransAM® NFκB p52 Chemi	1 x 96-well plate	48197
TransAM® NFκB p65	1 x 96-well plate	40096
TransAM® NFκB p65 Chemi	1 x 96-well plate	40097
TransAM® Flexi NFκB p65	1 x 96-well plate	40098
TransAM® Nrf2	1 x 96-well plate	50296
TransAM® Oct-4	1 x 96-well plate	42496
TransAM® p53	1 x 96-well plate	41196
TransAM® PPARγ	1 x 96-well plate	40196
TransAM® Sp1	1 x 96-well plate	41296
TransAM® Sp1/Sp3	1 x 96-well plate	40496
TransAM® STAT Family	2 x 96-well plates	42296
TransAM® STAT3	1 x 96-well plate	45196
TransAM® T-bet	1 x 96-well plate	51396

For a complete, up-to-date list of available TransAM® Kits, please visit **www.activemotif.com/transam**

Nuclear, Cytoplasmic and Whole-cell Extraction	Format	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
Recombinant GCN5 protein, active	5 µg	31204
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Histone Purification & Chromatin Assembly	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	10 rxns	40026
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

DNA Methylation	Format	Catalog No.
hMeDIP	10 rxns	55010
MeDIP	10 rxns	55009
MethylDetector™	50 rxns	55001
MethylCollector™	25 rxns	55002
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
DNMT Activity / Inhibition Assay	96 rxns	55006
Methylated DNA Standard Kit	3 x 2.5 µg	55008
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007

Co-Immunoprecipitation	Format	Catalog No.
Universal Magnetic Co-IP Kit	25 rxns	54002
Nuclear Complex Co-IP Kit	50 rxns	54001

SUMOylation	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

Recombinant Proteins	Format	Catalog No.
Recombinant c-Fos protein	5 µg	31115
Recombinant c-Jun protein	5 µg	31116
Recombinant c-Myc protein	5 µg	31117
Recombinant NFκB p50 protein	5 µg	31101
Recombinant NFκB p65 protein	5 µg	31102
Recombinant p53 protein	5 µg	31103
Recombinant p300 protein	4 µg	31124
Purified Sp1 protein	2 µg	31137
Recombinant STAT3 protein	10 µg	31140

For an up-to-date list of over 200 recombinant proteins, please visit **www.activemotif.com/proteins**.

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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