

TransAM™

Elk-1

Transcription Factor Assay Kits

(version A2)

Catalog Nos. 44396 & 44896

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Overview

Elk-1 is a transcription factor that is regulated by mitogen-activated protein kinases (MAPKs) and whose main function is the regulation of growth-related proteins, mainly c-Fos, in response to extracellular stimuli.¹ Because of the importance of Elk-1 to the regulation of successful cell growth, accurate monitoring of Elk-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.

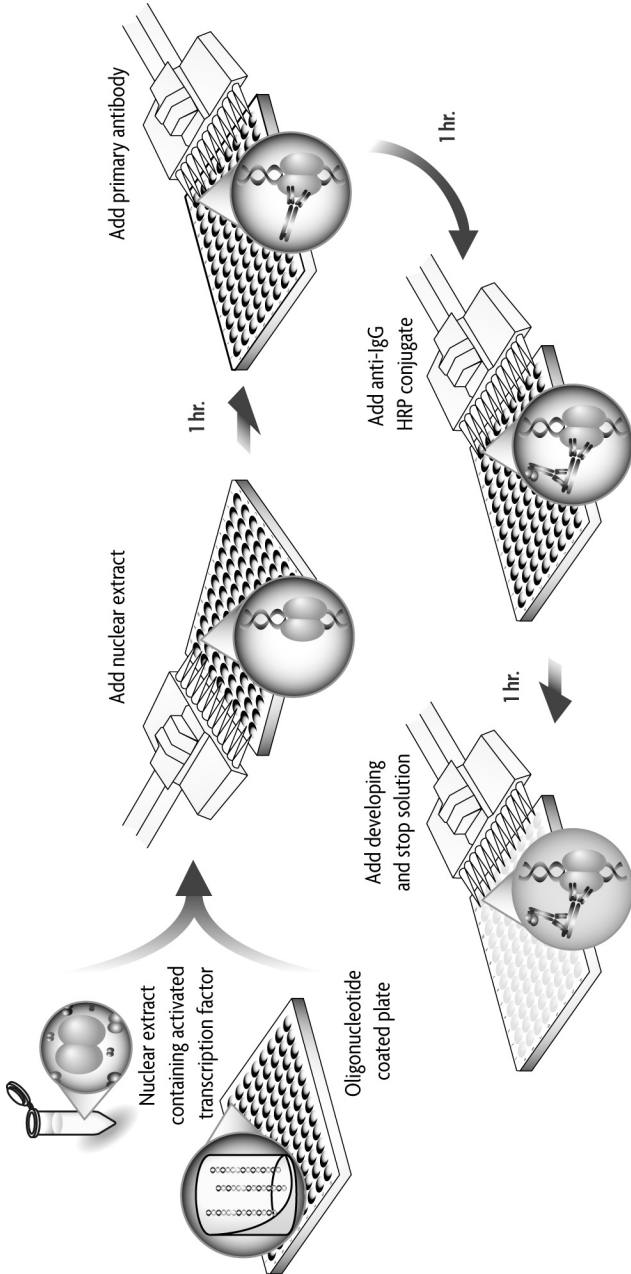
With its patented TransAM™ method*, Active Motif introduced the first ELISA-based kits to detect and quantify transcription factor activation. TransAM Kits combine a fast, user-friendly format with a sensitive, specific assay. TransAM Elk-1 Kits are designed specifically for the study of the MAPK regulated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

| product | format | catalog no. |
|---------------|--------------------|-------------|
| TransAM Elk-1 | 1 x 96-well plate | 44396 |
| | 5 x 96-well plates | 44896 |

Active Motif also offers the TransAM MAPK Family Kit that directly assays the transcription factors ATF-2, c-Jun, c-Myc, MEF2 and STAT1, all of which are regulated by MAP kinase cascades. See this and other Active Motif products related to Elk-1 in Appendix, Section B.

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

Flow Chart of Process



Introduction

Elk-1 Transcription Factor

The transmission of extracellular signals into intracellular responses is a complex process that often involves the activity of mitogen-activated protein kinases (MAPKs) (for review, see 2). The MAPK pathway is a three kinase cascade consisting of a MAPK kinase (MAPKKK or MEKK) that activates a MAP/ERK kinase (MEK or MAPKK). This stimulates a phosphorylation-dependent increase in the activity of the MAP kinase. Upon activation, MAPKs phosphorylate a variety of intracellular targets including transcription factors, transcription adaptor proteins, membrane and cytoplasmic substrates as well as other protein kinases.²

At least three parallel MAPK pathways exist in humans. The extracellular signal-regulated protein kinase (ERK) pathway primarily transmits mitogenic and differentiation stimuli, while the c-Jun N-terminal kinase (JNK) and p38 pathways predominantly transmit stress and cytokine stimuli.³

Elk-1 is a transcription factor that is activated by all three MAPK pathways. It is a member of the ternary complex factor (TCF) subgroup of the Ets protein family, along with Sap1 and NET/ERP/SAP2/Elk-3.⁴ Ets family members share an Ets domain and a winged helix-loop-helix (HLH) DNA binding domain that recognizes a GGAA/T-based sequence.^{4, 5} TCFs are able to form a ternary complex with the serum response factor (SRF) and the serum-response element (SRE), and are involved in SRE-driven gene expression.

Elk-1 has three major functional domains. The N-terminal Ets-DNA binding domain recognizes GGAA/T sequences. A motif of 20 amino acids, called the B domain, mediates protein-protein interactions with SRF. The C-terminal portion of Elk-1 has phosphorylation sites for ERK, JNK and p38 MAPKs. Elk-1 is mainly activated by ERK proteins, and binds to the SRE present in many immediate early genes such as *c-fos*, *egr1*, *egr2*, *pip92* and *nur77*.⁴

Transcription Factor Assays

To date, three methods are widely used to measure Elk-1, either directly or indirectly:

1. Expression can be measured by Western blot, using antibodies raised against Elk-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 Elk-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 Elk-1 binding. If Elk-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 Elk-1 activation is based on reporter genes, typically luciferase or β -galactosidase, placed under the control of a promoter containing the Elk-1 consensus sequence. This promoter can be artificial, made of several Elk-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 Elk-1

Elk-1 controls the activation of c-Fos and other immediate early genes and plays an important role in many areas of cell proliferation and survival. However, this field has been hampered by the lack of convenient, high-throughput assays suitable for large numbers of samples.

To overcome this, Active Motif is introducing a high-throughput assay to quantify the MAPK regulated transcription factor Elk-1. The TransAM Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for transcription factors. TransAM Elk-1 Kits contain a 96-well plate on which has been immobilized oligonucleotide that contains the consensus binding site for Elk-1. The active form of Elk-1 contained in the nuclear extract specifically binds to this oligonucleotide. The primary antibodies used to detect Elk-1 will recognize an epitope on phosphorylated Elk-1 that is accessible only when Elk-1 is activated and bound to the target DNA. 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 Elk-1 activation. With the 3.5-hour TransAM procedure, we could detect activation using as little as 1 μ g of nuclear extract.

TransAM has many applications including the study of drug potency, inhibitor or activator proteins, and protein structure/function studies in the Elk-1 signaling pathway.

Kit Performance and Benefits

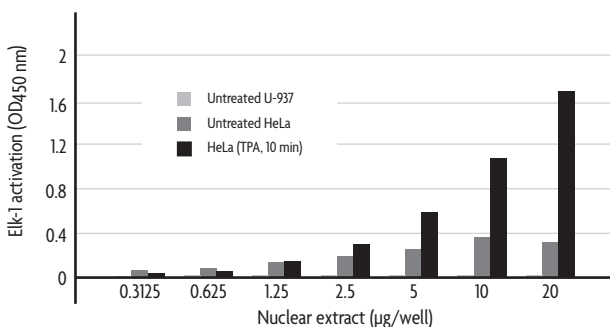
The TransAM Elk-1 Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: < 1.0 µg nuclear extract/well.

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

Cross-reactivity: TransAM Elk-1 detects phosphorylated Elk-1 (Ser 383) from human origin. Reactivity with other species has not been determined.

Assay time: 3.5 hours. TransAM is 40-fold faster than EMSA.



Monitoring Elk-1 activity in different cell lines with the TransAM Elk-1 Kit: Different amounts of nuclear extracts from untreated U-937, untreated HeLa and HeLa (TPA, 10 min) cells are tested for Elk-1 activity by using the TransAM Elk-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 |
|------------------------------------|--|------------------------------------|
| Phosphorylated Elk-1 antibody | 11 μl / 55 μl | 4°C for 6 months |
| Anti-mouse HRP-conjugated IgG | 11 μl / 55 μl (0.2 $\mu\text{g}/\mu\text{l}$) | 4°C for 6 months |
| Wild-type oligonucleotide AM4 | 100 μl / 500 μl (10 $\text{pmol}/\mu\text{l}$) | -20°C for 6 months |
| Mutated oligonucleotide AM4 | 100 μl / 500 μl (10 $\text{pmol}/\mu\text{l}$) | -20°C for 6 months |
| HeLa (TPA, 10 min) nuclear extract | 40 μl / 200 μl (2.5 $\mu\text{g}/\mu\text{l}$) | -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 |
| Poly [d(I-C)] | 100 μl / 500 μl (17 $\mu\text{g}/\text{ml}$) | -20°C for 6 months |
| Lysis Buffer AM3 | 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)

Protocols

Buffer Preparation and Recommendations

Preparation of Complete Lysis Buffer

We provide an excess of Lysis Buffer AM3 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 μ l of 1 M DTT and 10 μ l of Protease Inhibitor Cocktail per ml of Lysis Buffer AM3 (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 μ l of 1 M DTT and 10 μ l of 17 μ g/ml poly[d(I-C)] per ml of Binding Buffer AM2 (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Complete Binding Buffer.

Preparation of 1X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 100 ml of 1X Wash Buffer required, dilute 10 ml 10X Wash Buffer AM2 with 90 ml distilled water (see the Quick Chart for Preparing Buffers in this section). Mix gently to avoid foaming. The 1X Wash Buffer may be stored at 4°C for one week. The Tween 20 contained in the 10X Wash Buffer AM2 may form clumps, therefore homogenize the buffer by 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. 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.

HeLa nuclear extract

The HeLa (TPA, 10 min) nuclear extract is provided as a positive control for Elk-1 activation. Sufficient extract is supplied for 10 reactions. This extract is optimized to give a strong signal when used at 10 µg/well. We recommend aliquoting the extracts in fractions and storing at -80°C to help avoid multiple freeze/thaw cycles of the extracts. 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 Elk-1 binding in order to monitor the specificity of the assay. Used at 10 pmol/well, the oligonucleotide will prevent Elk-1 binding to the probe immobilized on the plate. Conversely, the mutated consensus oligonucleotide should have no effect on Elk-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 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 AM3 | 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 |
| | Poly[d(I-C)] | 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 mut oligo AM4 | 1 µl | 8 µl | 48 µl | N/A |
| | Complete Binding Buffer | 31.8 µl | 262 µl | 1.572 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 | 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.

Elk-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, 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, Washing Buffer, Antibody Binding Buffer, Developing Solution and Stop Solution into the wells being used.

Step 1: Binding of Elk-1 to its consensus sequence

1. Add 30 μ l Complete Binding Buffer to each well to be used. If you wish to perform competitive binding experiments, add 30 μ l Complete Binding Buffer that contains 10 pmol (1 μ l) 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 μ l of sample diluted in Complete Lysis Buffer per well. We recommend using 2-20 μ g of nuclear extract diluted in Complete Lysis Buffer per well. A protocol for preparing nuclear extracts is provided on page 11.

Positive control wells: Add 10 μ g of the provided nuclear extract diluted in 20 μ l of Complete Lysis Buffer per well (4 μ l of nuclear extract in 16 μ l of Complete Lysis Buffer per well).

Blank wells: Add 20 μ l 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 μ l 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 μ l diluted Elk-1 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 μ l 1X Wash Buffer (as described in Step 1, No. 4).

Step 3: Binding of secondary antibody

1. Add 100 μ l 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 μ l 1X Wash Buffer (as described in Step 1, No. 4).

Step 4: Colorimetric reaction

1. Add 100 μ l room-temperature Developing Solution to all wells being used.
2. Incubate 2-10 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 μ l 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 with 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 cm² (100 mm dish). The yield is approximately 0.5 mg of nuclear proteins for 10⁷ 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

0.1 M phosphate buffer, pH 7.5
1.5 M NaCl
27 mM KCl

For 250 ml, mix:

3.55 g Na₂HPO₄ + 0.61 g KH₂PO₄
21.9 g
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)

125 mM NaF
250 mM β-glycerophosphate
250 mM para-nitrophenyl phosphate (PNPP)
25 mM NaVO₃

For 10 ml, mix

52 mg
0.55 g
1.15 g
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)

20 mM HEPES, pH 7.5
5 mM NaF
10 μ M Na₂MoO₄
0.1 mM EDTA

For 50 ml, mix

0.24 g
12 mg
5 μ l of a 0.1 M solution
10 μ l 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 μ m filter. Store the filter-sterilized solution at 4°C.

References

1. Pust T. *et al.* (2002) *J. Biol. Chem.* 277(30): 27517-27527.
2. Pearson G. *et al.* (2001) *Endocrine Rev.* 22(2): 153-183.
3. Yang S.H. *et al.* (1998) *EMBO J.* 17: 1740-1749.
4. Vanhoutte P. *et al.* (2001) *J. Biol. Chem.* 276(7): 5189-5196.
5. Lehmann U. *et al.* (1999) *J. Biol. Chem.* 274(3): 1736-1744.

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 5 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 to 20 µg/well |
| | Elk-1 is poorly activated or inactivated | Perform a time course for Elk-1 activation in the studied cell line |
| | Cell extracts are not from correct species | Refer to cross-reactivity information on page 5 |

Section B. Related Products

| Transcription Factor ELISAs | Unit | Catalog No. |
|-----------------------------|-------------|-------------|
| 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™ MAPK Family | 2 x 96 rxns | 47296 |
| TransAM™ MEF2 | 1 x 96 rxns | 43196 |
| | 5 x 96 rxns | 43696 |
| TransAM™ STAT Family | 2 x 96 rxns | 42296 |
| TransAM™ STAT3 | 1 x 96 rxns | 45196 |
| | 5 x 96 rxns | 45696 |

| Cell-based ELISAs | Unit | 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™ EGFR | 1 x 96 rxns | 48150 |
| | 5 x 96 rxns | 48650 |
| FACE™ EGFR Chemi | 1 x 96 rxns | 48250 |
| | 5 x 96 rxns | 48750 |
| 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™ 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™ p38 | 1 x 96 rxns | 48100 |
| | 5 x 96 rxns | 48600 |
| FACE™ p38 Chemi | 1 x 96 rxns | 48200 |
| | 5 x 96 rxns | 48700 |

| 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 |

| Cell extracts | Unit | Catalog No. |
|------------------------------------|----------|-------------|
| Nuclear Extract Kit | 100 rxns | 40010 |
| | 400 rxns | 40410 |
| Mitochondrial Fractionation Kit | 100 rxns | 40015 |
| HeLa (TPA, 10 min) nuclear extract | 200 µg | 36109 |
| HeLa (Anisomycin) nuclear extract | 200 µg | 36111 |

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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Carlsbad, CA 92008

USA

Toll Free: 877 222 9543

Telephone: 760 431 1263

Fax: 760 431 1351

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France Free Phone: 0800 90 99 79

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