Instruction Manual
for

Universal EZ-TFA

Transcription Factor Assay Chemiluminescent

Catalog # 70-601

Sufficient reagents for 192 reactions.

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I. INTRODUCTION

The UPSTATE® Non-Radioactive Universal EZ-TFA Transcription Factor Assay provides a fast, sensitive method to detect specific transcription factor DNA binding activity in whole cell or nuclear extracts. This assay combines the principle of the electrophoretic mobility shift assay (EMSA) with the 96-well based enzyme-linked immunosorbent assay (ELISA), enabling manual or high-throughput sample analysis with greater sensitivity than conventional EMSA assays. After certain parameters are optimized, the entire assay takes less than 4 hours to complete with minimal hands-on time. The versatile set up allows for a flexible assay design and because the binding reaction occurs in solution, various parameters can be optimized such as probe titration, competitive oligonucleotide concentration, or treatment conditions. This kit contains the reagents and instructions necessary to develop an assay to monitor the DNA binding activity of any transcription factor of interest, or as a kit component for Upstate developed target specific Transcription Factor Assays. The Universal kit also allows for the design of native promoter capture probe sequences to interrogate specific transcription factor binding.

II. TEST PRINCIPLE

The Non-Radioactive Universal Transcription Factor Assay kit is provided in a 96-well format. During the assay, the capture probe, a double stranded biotinylated oligonucleotide containing the consensus sequence for the specific transcription factor under study, is mixed with cellular (nuclear) extract in the transcription factor assay buffer provided directly in the streptavidin coated plate. When incubated together, the transcription factor contained in the extract binds to its consensus sequence. The biotinylated double stranded oligonucleotide bound to the transcription factor being studied is immobilized on the streptavidin-coated plate and any inactive, unbound material is washed away. The bound transcription factor is detected with a specific primary antibody. An HRP-conjugated secondary antibody is then used for detection and provides sensitive chemiluminescent detection that can be read in a microplate luminometer or by a CCD camera-coupled imaging system.

Kit Description

Quantity: One box containing the necessary reagents to perform 192 Reactions.

Storage and Stability: Store kit components at the temperatures indicated on the labels. When handled as described in this manual, the materials in this product are stable for 6 months from date of shipment.

III. UNIVERSAL TFA KIT COMPONENTS

A. Provided Kit Components (Note Storage Temperatures)

Store at 4°C:

Streptavidin Coated Chemiluminescent Plate, Catalog # 90494. Two white 96-well Streptavidin-coated plate with strip wells sealed in a foil pouch with desiccant.

Blocking Reagent, Catalog # 2003488. One vial containing 24 grams of Blocking Reagent.

10X Annealing Buffer, Catalog # 2007044. One vial containing 250 µL 10X Annealing Buffer to be used in the preparation of biotinylated capture probe and specific competitor.

Chemiluminescent Detection Reagent, Catalog #90495. Two bottles containing 4 mL of Chemiluminescent Detection Reagent.

Chemiluminescent Reaction Buffer, Catalog #90496. Two bottles containing 8 mL of Chemiluminescent Reaction Buffer.

Store at -20°C:

TFA Buffer, 5X, Catalog # 2003487. One bottle containing 125 mL of 5x Transcription Factor Assay Buffer containing sonicated salmon sperm DNA to block non-specific DNA binding activity.

Gt X Rb, HRP Secondary Antibody, Catalog # 2003494. One vial containing 60 µL Goat anti-Rabbit, HRP conjugated secondary detection antibody. Use at 1:500 dilution in Enhanced Transcription Factor Assay Buffer.

Gt X Ms, HRP Secondary Antibody, Catalog # 2003482. One vial containing 60 µL Goat anti-Mouse, HRP conjugated secondary detection antibody. Use at 1:500 dilution in Enhanced Transcription Factor Assay Buffer.

B. Required Materials Not Provided

Reagents

- Cells, stimulated or treated as needed for the experimental system
- Capture probe
- Specific competitor
- Primary antibody
- Nuclear extraction kit (Catalog # 2900)
- Nuclease free water (Catalog # 3098)

Equipment

- Heating Block
- Luminometer

IV. SAMPLE PREPARATION

(Performed Prior to Assay)

Cytosolic and Nuclear Extraction Procedure

Prior to performing the Upstate® EZ-TFA Transcription Factor Assay, nuclear or whole cell protein extraction is required. To perform a nuclear extraction, we recommend using Chemicon's Nuclear Extraction Kit (Cat. No. 2900), or for a quick procedure, Chemicon offers a Whole Cell Extraction kit (Cat. No. 2910).

If the above referenced kits are not used to perform the protein extraction, the following procedure is recommended. Various other methods could be utilized to prepare cytosolic and nuclear extracts, though the following recommended protocol has been tested and works well with the Upstate Transcription Factor Assay.

Materials Required for Nuclear Extraction:

1. PBS (pH 7.5)
2. Protease Inhibitor Cocktail (in DMSO)
3. Syringe with No. 27 needles (small gauge)
4. Materials for buffers: HEPES, $MgCl_2$, KCl, EDTA, DTT, Triton X-100, NaCl, glycerol, and Igepal CA-630 (NP-40 can be used in place of Igepal CA-630).

Make Buffer A and Buffer B required for the nuclear extraction in advance. Store both buffers on ice. ***Note:*** *Fresh DTT and protease inhibitors should be added just prior to use.*

Buffer A (Hypotonic Lysis Buffer)

10 mM HEPES (pH 7.9)
1.5 mM $MgCl_2$
10 mM KCl
0.5-5 mM DTT
0.1% Triton X-100
Protease inhibitor cocktail

Note: *Buffer A can be made at a 10x concentration and stored at 2-8^oC without the DTT and Protease inhibitors added.*

Note: *Increased amounts of DTT may be necessary dependent upon cell type, stimulation and the transcription factor studied.*

Buffer B (Extraction Buffer)

20 mM HEPES (pH 7.9)
1.5 mM $MgCl_2$
0.42 M NaCl
0.2 mM EDTA
0.5-5 mM DTT
1.0% Igepal CA-630
25% (v/v) glycerol
Protease inhibitor cocktail

Note: *All work done after cell trypsinization/detachment needs to be performed on ice and/or with chilled buffers. It is imperative that the cell pellets and suspension remain as cold as possible without freezing during the extraction process.*

Note: *Increased amounts of DTT may be necessary dependent upon cell type, stimulation and the transcription factor studied.*

A. Cell Culture

1. Grow cells to desired confluency and add appropriate activators and/or inhibitors.

B. Cell Disruption

1. For adherent cells, wash the cells with HBSS (Hank's Balanced Salt Solution) or PBS and then add warmed trypsin to the culture flask(s). Let the trypsin sit for about 2 minutes and shake the cells off. Alternately, cell lifters may be used instead of trypsin. Collect cells and transfer them to a clean centrifuge tube, rinsing the culture flask with ice cold PBS. Centrifuge the sample at 250 x g for 5 minutes. Discard the supernatant and resuspend the cell pellet in 40 mL of ice-cold PBS. Centrifuge the suspension at 250 x g as before. Repeat. Pour off supernatant.
2. Estimate the approximate volume of the centrifuged cell pellet. This value will be needed for determining the amount of the various buffers to add for nuclear extraction.
3. Add 5 cell pellet volumes of ice cold Buffer A (lysis buffer).
4. Resuspend the cell pellet by gently inverting the tube avoiding foam production. **Do not vortex.**
5. Incubate the cell suspension in ice cold Buffer A for 15 min on ice.
6. Centrifuge the cell suspension for 5 minutes at 250 x g. Discard supernatant and resuspend the cell pellet in two volumes of ice cold Buffer A.

C. Cell Lysis

Note: *All subsequent steps must be performed on ice.*

1. Using a syringe with a small gauge needle (a No. 27 gauge needle works well) slowly draw the cell suspension prepared in Section B, Step 6 into the syringe and then eject the contents with a single stroke. Repeat approximately 5 times (drawing and ejecting). If the cells "clump" and you are not able to draw them into the syringe with a No. 27 gauge needle, more non-ionic detergent (Igepal CA-630 or NP40) may need to be added.
2. Centrifuge the disrupted cell suspension at 8,000 x g for 20 minutes.
3. The supernatant contains the cytosolic portion of the cell lysate. Transfer the supernatant to a fresh tube. To keep the cytosolic fraction, snap-freeze and store in aliquots at –70°C. Avoid repeated freeze-thaw cycles.
4. The remaining portion (pellet) contains the nuclear portion of the cell lysate.

D. Nuclear Extraction

1. Resuspend the nuclear pellet in 2/3 of the original cell pellet volume (determined in step B.2) ice cold Buffer B.
2. Using a fresh syringe (with a No. 27 gauge needle), repeat Step C.1. to disrupt the nuclei.

   Note: *The nuclear extract sample can be stored at –80°C at this point if needed.*
3. Use a rotator (low speed) to gently agitate the nuclear suspension at 4°C for 30-60 minutes.
4. Centrifuge the nuclear suspension at 16,000 x g for 5 minutes at 4°C.
5. Transfer the supernatant to a fresh tube. This fraction is the nuclear extract.
6. Determine protein concentration and adjust the final protein concentration to 2.5 to 5 mg/mL with ice cold PBS.
7. Snap-freeze the nuclear extract in aliquots and store at –80°C. Avoid repeated freezing and thawing of nuclear extract.

V. ASSAY DEVELOPMENT

A. Design and production of biotinylated capture probe – Oligonucleotide Annealing

1. Determine the specific consensus sequence of the transcription factor being studied. Once the consensus sequence has been identified, synthesize two complementary oligos at least 50 nucleotides in length that contain the sequence of interest.
    a. The sense strand oligo should contain a 5'-end biotin modification and the consensus sequence should be on the opposite 3' end of the oligo. There should also be at least 5-10 nucleotides to the 3'-end of the consensus sequence flanking the binding site of interest.
    b. The complementary antisense oligo should not contain any end-modifiers.
2. Resuspend each oligo to 10 pmol/µL in nuclease free water.
3. Preheat a heatblock to 95°C.
4. Combine the following reagents in the order listed in a 1.5 mL microcentrifuge tube.
    a. 175 µL of nuclease free water
    b. 25 µL of 10X Annealing Buffer
    c. 25 µL of 10 pmol/µL 5'- biotin labeled sense strand oligonucleotide containing consensus sequence
    d. 25 µL of 10 pmol/µL unlabeled antisense oligonucleotide
5. Heat the oligonucleotide mixture to 95°C for 10 min.
6. Switch off the heat block and allow the mixture to equilibrate to room temperature (about 1-2 hours).
7. Store the capture probe (1 pmol/µL) in usable aliquots at -20°C.

B. Design and production of biotinylated capture probe – PCR

1. As an alternative to the oligo heat annealing procedure, the capture probe containing the specific consensus sequence for transcription factor binding can be generated via PCR.
    a. This would be done by generating 3 synthetic oligonucleotides.
        I. The first one is the amplification template that will be used by a 5' biotinylated primer containing a tail sequence and a 3' primer that contains the consensus sequence that is flanked on either side by at least 5 bps. This amplification template includes a 50-100 bp spacer at the 5' end that does not include any potential transcription factor binding sites. This sequence is used to tether the capture probe to the plate and to allow the transcription factor to bind to the consensus sequence. The 3' end of this amplification template includes the transcription factor binding consensus sequence site with at least 5 bps on either side of the consensus sequence.
        II. The second oligonucleotide is the biotinylated 5' primer. This is 5'-end biotin modified sense oligo that is used to create the bioylated capture probe.
        III. The third oligonucleotide is the 3' unmodified antisense oligo flanking the binding site of interest with at least 5 mucleotides 3' to the consensus sequence.
        IV. The resulting PCR product should have the transcription factor binding site at the 3' end distal to the biotin modifier.

b. The final product should be run on a gel to ensure that the PCR reaction was successful. If the product is a good single band at the correct size, the final PCR product should be purified and diluted to a final concentration of 1µM in TE, pH 7.

C. Design and production of specific competitor

1. Design two complementary oligonucleotides that contain the exact same sequence as the capture probe but do not contain any biotin modifications. These oligos can be significantly shorter than the capture probe as long as they contain at least 5-10 nucleotides flanking the consensus sequence.
2. Resuspend each oligo to 100 pmol/µL in nuclease free water.
3. Preheat a heatblock to 95°C.
4. Combine the following reagents in the order listed in a 1.5 mL microcentrifuge tube.
    a. 35 µL of nuclease free water
    b. 5 µL of 10X Annealing Buffer
    c. 5 µL of 100 pmol/µL sense strand oligonucleotide containing consensus sequence
    d. 5 µL of 100 pmol/µL antisense oligonucleotide
5. Heat the oligonucleotide mixture to 95°C for 10 min.
6. Switch off the heat block and allow the mixture to equilibrate to room temperature (about 1-2 hours).
7. Store the specific competitor (10 pmol/µL) at -20°C.

D. Design and production of Negative Control Probe

1. A negative control probe should be designed to control for non-specific binding to the capture probe. This probe must consist of the same sequence as the capture probe, but not contain the binding site of interest, or contain a specific binding site mutation or randomly scrambled sequence.
    a. Design two complementary oligonucleotides that have the exact same sequence as the capture probe but do not contain a functional binding site. The sense strand oligonucleotide should have a 5'-end biotin modification.
    b. The complementary antisense oligonucleotide should not have any end-modifiers.
    c. If the PCR method was followed to create the capture probe, a negative probe containing the tail sequence should be generated as well to ensure that there was not any transcription factor binding to this region.
2. Resuspend each oligo to 20 pmol/µL in nuclease free water.
3. Preheat a heatblock to 95°C.
4. Combine the following reagents in the order listed in a 1.5 mL microcentrifuge tube.
    a. 35 µL of nuclease free water
    b. 5 µL of 10X Annealing Buffer
    c. 5 µL of 20 pmol/µL sense strand oligonucleotide containing consensus sequence
    d. 5 µL of 20 pmol/µL antisense oligonucleotide

5. Heat the oligonucleotide mixture to 95°C for 10 min.
6. Switch off the heat block and allow the mixture to equilibrate to room temperature (about 1-2 hours).
7. Store the negative control probe (2 pmol/µL) at -20°C.

E. Selection of appropriate primary antibody

1. In order to select a functional primary antibody to detect the DNA binding activity of the transcription factor of interest, it is best to consider the following:
   a. A highly characterized antibody that performs well in gel supershift assays is preferred.
   b. An antibody that has been validated in ChIP assays may also perform well in this assay.
   c. The antigen of the primary antibody should not be physically proximal to the DNA binding domain, or should not occur near a region where antibody binding may compromise a required protein:DNA or protein:protein interaction surface.
2. The initial antibody titration should be done using a positive control cell or nuclear extract known to contain active transcription factor, or can alternately be performed using active recombinant purified protein. Determining the optimal primary antibody concentration is critical to the assay development process. Suggested starting dilutions for the primary antibody are 1:100 – 1:1000, but are dependent on quality and concentration of the primary antibody being employed, as well as the concentration of antigen present in the positive control sample.

VI. ASSAY PROTOCOL

A. Prepare 1x Transcription Factor Assay Buffer (TFA Buffer)

1. Calculate the total volume of 1x TFA Buffer required: Each well requires 2.7 mL of 1x TFA Buffer (i.e. 8 wells require 21.6 mL of 1x TFA Buffer).
2. Dilute 1 part 5x TFA Buffer into 4 parts distilled water to obtain the desired amount of buffer.

B. Prepare Enhanced Transcription Factor Assay Buffer (Enhanced TFA Buffer)

1. Take a portion of the 1x TFA Buffer prepared in Step A and use to prepare 1x Enhanced TFA Buffer by adding blocking reagent.
2. Calculate the total volume of Enhanced TFA Buffer required for the assay run: Each well requires 1.5 mL of 1x TFA Buffer supplemented with 0.09 g Blocking Reagent (i.e. 8 wells require 12 mL of enhanced TFA buffer made with 0.72 g of blocking reagent). The Enhanced TFA Buffer will be used for all steps of the assay with the exception of the final wash following secondary antibody incubation. For this wash, use the remaining 1x TFA Buffer prepared in Step A (approximately 1.2 mL per assay/well).
3. Mix the Blocking Reagent with 1x TFA Buffer making sure that the blocking reagent is completely dissolved in the TFA buffer prior to starting the assay.

C. Sample Incubation

1. Prepare the test sample, negative control sample, positive control sample, and/or competitive oligonucleotide control directly in the plate wells as described in the table below.

Add the components in sequential order as follows:

1st. 1x Enhanced TFA Buffer

2nd. Oligonucleotide probes

3rd. Cell or nuclear extract

Note: *Sample extract must always be added to the binding reaction mixture last.*

Note: *Never use excessive heat to thaw the capture probes or competitor oligonucleotide. Use of heat can destroy product.*

	Enhanced Transcription Factor Assay Buffer (1x)	**Capture Probe**	**Competitor Oligonucleotide**	**Negative Control Probe**	**Nuclear Extract**	**Total Volume**
Transcription Factor Assay (Normal)	47μL	2μL	–	–	1μL (2.5μg/μL)	50μL
Transcription Factor Assay—Positive Control	47μL	2μL	–	–	1μL (2.5μg/μL)	50μL
Transcription Factor Assay—Specific Competitor Control	45μL	2μL	2μL	–	1μL (2.5μg/μL)	50μL
Transcription Factor Assay—Negative Control	48μL	–	–	1μL	1μL (2.5μg/μL)	50μL

a. Positive Control:

A positive control extract should be made that is known to express the specific transcription factor being studied and be treated in a way to induce its' transcriptional activity. Obviously, if the transcription factor is not well characterized, this control is difficult to produce. Another option would be to run recombinant transcription factor as positive control.

Note: *The chemiluminescent detection system used in this assay is extremely sensitive, therefore it may be necessary to titrate the positive control extract to determine an optimal concentration. If the protein concentration is too high, the background noise will also be high.*

b. Competitor Control:

The specific competitor control oligonucleotide is included in the assay to ensure that the complex is binding the probe DNA in a sequence specific manner. It is an unlabeled competitor oligonucleotide containing the identical consensus sequence as the capture probe. This control will compete with the capture probe for transcription factor binding. With a typical nuclear extract, this assay setup should greatly diminish the signal intensity. However, assays may be performed using varying amounts of the capture probe and/or specific competitor oligonucleotide as desired. Alternatively,

the investigator may choose to incorporate other competitor oligonucleotides into the assay. *Keep in mind that any increase in volume of added competitor oligonucleotide must be compensated for by an adjustment in the amount of 1x TFA Buffer in the assay set up such that the final volume equals 50µL.*

c. **Negative Control:**

The Negative Control Probe is used without the addition of the Capture Probe. The Negative Control Probe is used to ensure that the signal obtained from the normal assay setup is specific to the transcription factor binding to its consensus sequence. Any signal obtained with this assay setup will reflect non-specific binding.

2. Incubate in plate well for 1-2 hours at room temperature. A 1 hour incubation is sufficient in most cases, but a 2 hour incubation can result in a slightly higher overall signal as well as a slightly higher signal to noise ratio, especially when using less sample. Cover the sample wells during this and all subsequent non-wash incubations.

3. Wash wells three times with 150 µL of the 1x Enhanced TFA Buffer. Incubate each wash as follows: 1st wash, 30 seconds; 2nd wash, 1 minute; 3rd wash, 3 minutes. Following each wash, invert the plate over a basin to empty the wells and gently tap the inverted plate a few times onto an absorbent pad. Completely remove the buffer from the wells following the final wash.

Note: *Mild agitation or rotating during this and all subsequent washes may give cleaner results, but is not necessary.*

D. Primary Antibody Incubation

1. Dilute primary antibody in 1x Enhanced Transcription Factor Assay Buffer to the determined concentration from the initial antibody titration (as discussed above).

   a. Prepare enough for 100 µL of diluted antibody per assay well tested**.**

2. Add 100 µL of diluted primary antibody to each assay well. Incubate for 60 minutes at room temperature.

3. Wash wells three times with 150 µL of the 1x Enhanced Transcription Factor Assay Buffer (as in step C.3.). Incubate each wash as follows: 1st wash, 30 seconds; 2nd wash, 1 minute; 3rd wash, 3 minutes. Completely remove the buffer from the wells following the final wash.

E. Secondary Antibody Incubation

1. Dilute the appropriate IgG-HRP conjugated secondary antibody in 1x Enhanced Transcription Factor Assay Buffer at a 1:500 dilution.

   a. Prepare enough for 100 µL of diluted secondary antibody per assay well tested.

2. Add 100 µL of the diluted secondary antibody to each well and incubate at room temperature for 30 minutes.

3. Wash wells four times with 250 µL of 1x Transcription Factor Assay Buffer **(WITHOUT BLOCKING REAGENT)** per well. Incubate each wash as follows: 1st wash, 30 seconds; 2nd wash, 2 minutes; 3rd wash, 3 minutes; 4th wash, 4 minutes.

Note: *Using 1x Enhanced TFA Buffer, which contains Blocking Reagent, in this step will increase background signal.*

F. Chemiluminescent Development

1. Add 1 part Chemiluminescent Detection Reagent to 2 parts Chemiluminescent Reaction Buffer making enough to add 100 µL/well.
2. Add 100 µL of the pre-mixed chemiluminescent substrate solution to each assay well being tested. The development time may vary according to laboratory conditions.
3. Measure the luminescence of samples according to the directions given with the microplate luminometer (typically 0.2-1.0 seconds integration time) or the CCD camera coupled imaging device.

VII. CALCULATION OF RESULTS

Relative light unit (RLU) values obtained using UPSTATE's® EZ-TFA Universal Transcription Factor Assay Kit may be compared with known standards or other test samples to obtain relative activities. The following graph represents data obtained by using the Universal Transcription Factor Assay. The data below is for reference use only and the data should not be used to interpret actual assay results.

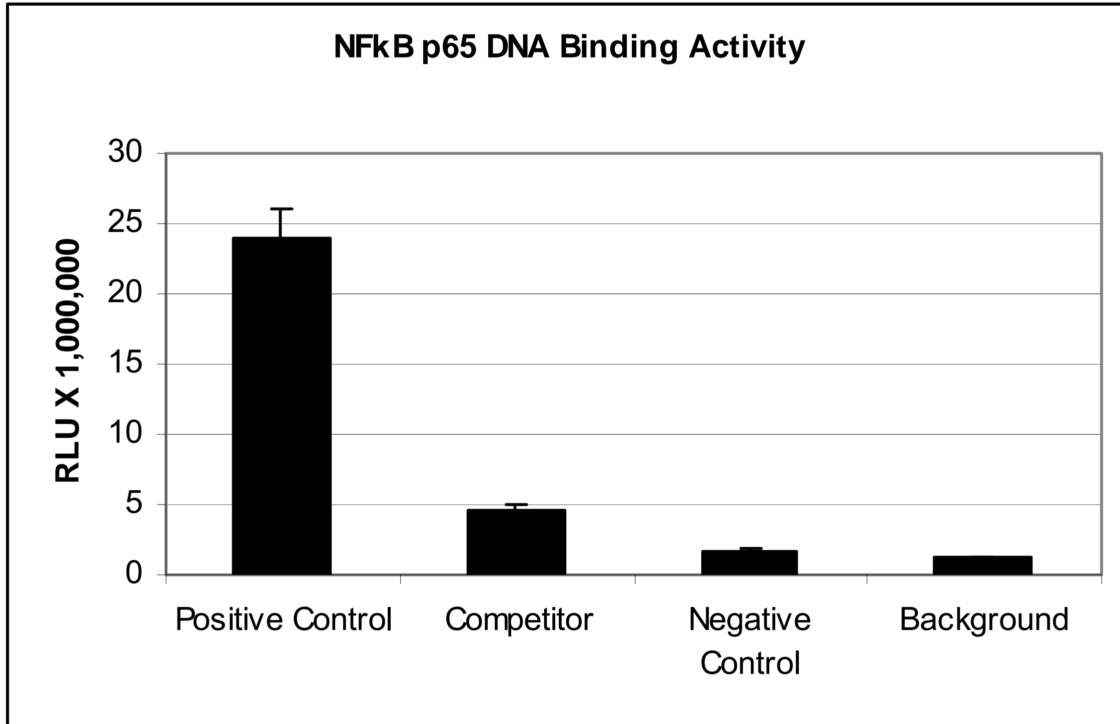

Figure 1: Example of Universal Transcription Factor Assay using annealed oligo NFκB capture probe, NFκB Competitor, negative control probe, p65 primary antibody, and TNFα treated HeLa whole cell extract.

VIII. TROUBLESHOOTING

1. Weak or no signal in all wells

Cause	Solution
Improper primary antibody dilution	Re-titrate primary antibody to obtain a more highly concentrated working solution.
"Positive control" extract is not appropriate	Use cells known to express the transcription factor of interest and appropriate treatments to induce DNA binding activity.
Extract concentration is too low	Increase "positive control" extract used in the binding reaction. Suggested amount of extract is between 5 µg and 15 µg.
Improper preparation or storage of a reagent	Make sure that all reagents are stored at their proper temperatures. Avoid repeat freeze/thawing of frozen materials.
Reagent Expired	Check the date on reagents to make sure they have not expired.
Insufficient cell extract per well resulting in low sample concentration	Perform cell extract titer to determine optimal concentration. The Enhanced Transcription Factor Buffer is tolerant of variations in sample volume.
Salt concentrations affecting DNA: protein binding	Reduce the amount of extract used in assay or reduce the amount of salt in extraction buffer. Alternatively, perform buffer exchange.
Plate reader not working well	Allow luminometer to warm up. Check Plate reader.

2. High signal in all wells

Cause	Solution
Improper antibody dilution	Retitrate the primary antibody to obtain a more dilute working solution.
Extract concentration is too high	Decrease the amount of extract in the binding reaction. Suggested amount of extract is between 0.5 µg and 2.5 µg.
Improper or inadequate washing of the wells	Follow the protocol as to the times and volume of each wash. Do not use 1x Enhanced Transcription Factor Assay Buffer for the final wash. 1x Transcription Factor Assay Buffer without Blocking Reagent should be used for the final wash.

3. High background in sample wells

Cause	Solution
Sample concentration to high	Adjust the concentration by generating a dose response curve with your sample to determine proper concentration of sample to use.
Improper antibody dilution	Retitrate the primary antibody to obtain a more dilute working solution.

4. Weak signal in sample wells

Cause	Solution
Sample concentration too low	Not enough cellular extract was used. Increase volume, or prepare fresh sample as weak signal can result from poor sample preparation.

IX. Related Products

Universal EZ-EZ-TFA Transcription Factor Assays

70-500 Universal EZ-EZ-TFA Transcription Factor Assay, Colorimetric
70-600 Universal EZ-EZ-TFA Transcription Factor Assay, Chemiluminescent
70-501 Universal EZ-TFA Transcription Factor Assay, Colorimetric
70-601 Universal EZ-TFA Transcription Factor Assay, Chemiluminescent

Target Specific Kits:

Each target specific kit includes either the colorimetric or chemiluminescent Universal kit module as well as all of the necessary antibodies, probes, controls, and instructions to properly and easily run the target specific assay.

Target	Colorimetric	Chemiluminescent
NFκB Family (p50, p52, p65 (RelA), RelB, c-Rel)	70-560	70-660
NFκB, p50/p65	70-510	70-610
NFκB, p50	70-515	70-615
NFκB, p65	70-520	70-620
AP-1 Family (Jun, JunB, JunD, Fos, FosB, Fra-1, Fra-2)	70-550	70-650
Jun/Fos	70-546	70-646
c-Jun	70-540	70-640
c-Fos	70-545	70-645
STAT1α	70-535	70-635
STAT3	70-530	70-630
p53	70-525	70-625
FOX01	70-555	70-655
CREB	70-575	70-675
Oct-4	70-565	70-656
HIF-1α	70-570	70-670

Antibodies and Reagents

Please refer to our website or Transcription brochure for the most up to date list of our extensive product offering for transcription research.