RayBio® Human ELK-1 Transcription Factor Activity Assay Kit

Catalog #: TFEH-ELK1

User Manual
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3607 Parkway Lane, Suite 200
Norcross, GA 30092
Tel: 1-888-494-8555 (Toll Free) or 770-729-2992, Fax: 770-206-2393
Web: www.RayBiotech.com, Email: info@raybiotech.com

RayBiotech, Inc.

RayBio® Human ELK-1 TF Activity Assay Kit Protocol

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Please read the entire manual carefully before starting your experiment.

I. INTRODUCTION

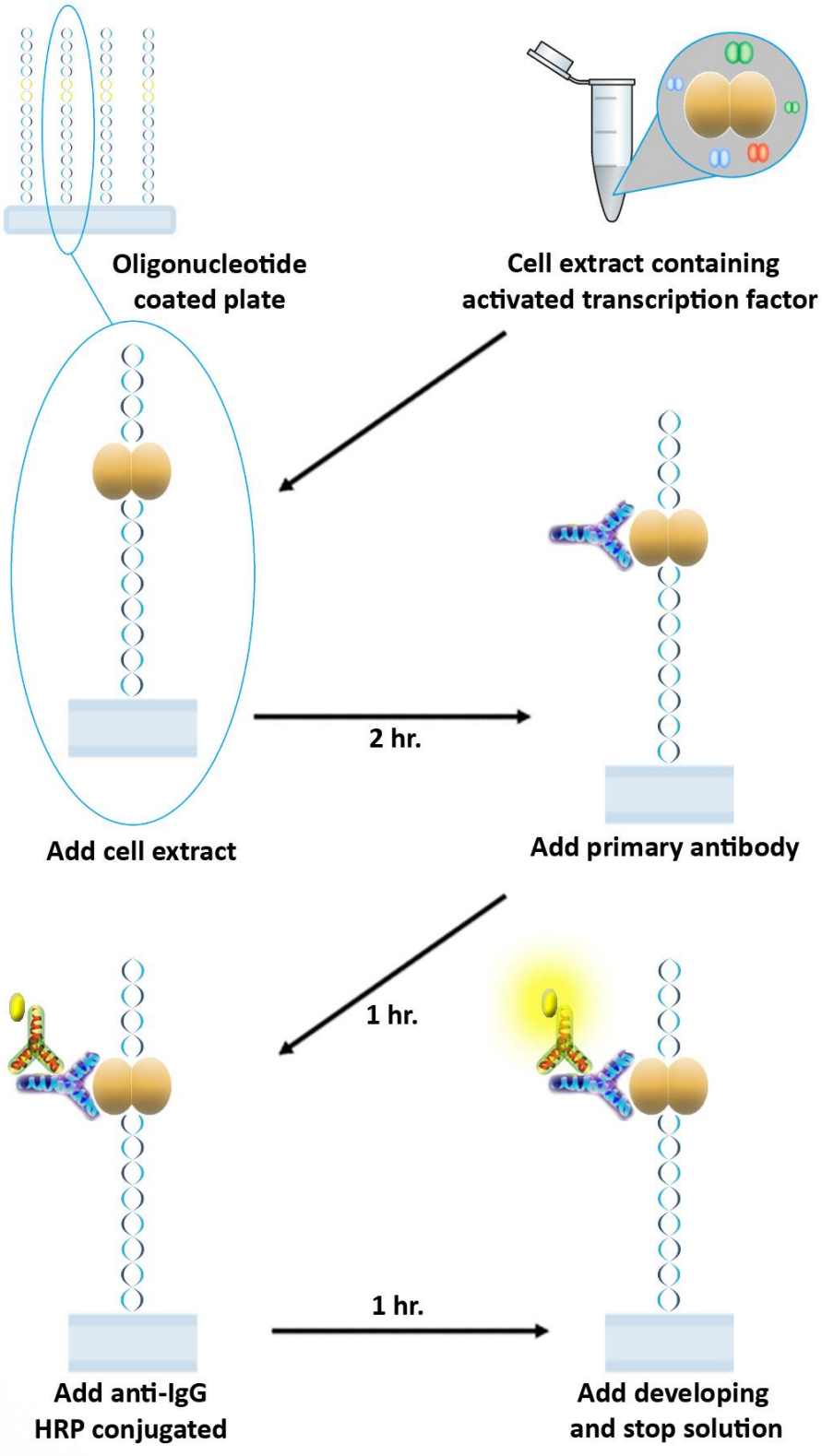
MAP kinase (MAPK) pathways including ERK1/2, p38 and JNK are activated upon environmental stimulus by growth factors or cellular stress, such as UV irradiation. This signaling serves to transmit external stimuli to the nucleus and activate numerous transcription factors. This results in changes in gene expression required for cellular growth, differentiation and survival. One downstream effector of these signaling pathways is Elk-1, a transcription factor that regulates immediate early gene (IEG) expression via the serum response element (SRE) DNA consensus site. Elk-1 belongs to the ternary complex factor (TCF) subfamily, part of Ets (E twenty-six) oncogene family of transcription factors which contain a highly conserved DNA-binding domain (DBD, the Ets domain) comprised of 85 amino acids. The TCF subfamily consists of three members ELK-1, SAP-1 and SAP-2/Net which are distinct gene products with different chromosomal localizations in both the mouse and human genomes. Each of the TCFs contains three conserved domains including the N-terminal Ets DNA binding domain, B-box region engaged in the interaction with serum response factor (SRF) to form ternary complex, and C-terminal transcriptional activation domain. Elk-1 can be activated by either pathway of ERK, JNK, or p38 through phosphorylation and de-SUMOylation. SUMO modification of Elk-1 by ERK pathway represses its transcriptional activity via SUMO dependent recruitment of histone deacetylases. Phosphorylated and de-sumoylated ELK-1 translocates to the nucleus from the cytoplasm and binds to SRE DNA sequences containing a central GGAA/T motif to initiate expressions of involved genes.

Accurate monitoring of the level of activated ELK-1 in cells, tissues, or animal models is required for the investigation of signal transduction pathways both in basic science research and applications such as drug development. As such, there is a significant need for a simple, speedy and high-throughput method for this purpose.

Traditionally, western blotting to detect the expression and modification of ELK-1, electrophoretic mobility shift assay (EMSA) to detect the DNA binding capacity of ELK-1, or transfection of reporter genes such as luciferase and β-galactosidase with ELK-1 binding sites in culture cells are used in the evaluation of ELK-1 reactivity. However, these methods are time consuming, laborious, and sometimes require the use of radioactivity.

The RayBio® ELK-1 TF Activity Assay kit is a non-radioactive transcription factor assay with an ELISA format. It offers an easy, quick, sensitive, and high-throughput method to detect the activation of transcription factors. In 96 well plates, double stranded oligonucleotides containing ELK-1 binding sequence have been coated. These oligonucleotides specifically capture the active ELK-1 contained in whole cell lysates or nuclear extracts after a short incubation. Subsequently, the primary antibody against ELK-1 recognizes the ELK-1-DNA complex in each well, and a HRP-conjugated secondary antibody is then used for detection. After washing away any unbound antibody, signal can be obtained easily through a colorimetric assay with a spectrophotometric plate reader at 450 nm. The specificity of the reaction between active ELK-1 and the DNA probe is additionally stringent because of the establishment of specific competitive DNA and non-specific competitive DNA probes in this reaction system.

II. HOW IT WORKS



III. STORAGE

Upon receipt, the positive control should be removed and stored at -20° or -80°C. The remainder of the kit can be stored for up to 6 months at 2-8°C from the date of shipment. Opened Microplate Wells or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
Note: The kit can be used within one year if the whole kit is stored at -20°C upon receipt. Avoid repeated freeze-thaw cycles.

IV. REAGENTS

Component	Description	Size
ELK-1 DNA Probe Microplate	96 wells (12 strips X 8 wells) coated with ELK-1 probes	1 plate
DNA Binding Buffer	5X concentrated Buffer	4 ml
Positive Control	Cell nuclear extracts	1 vial (20 µl)
Specific Competitor DNA Probe	Free DNA probes that compete with the coated probes by binding with activated ELK-1.	1 vial
Non-specific Competitor DNA Probe	Free DNA probes with mutations of the coated DNA probe. Cannot bind activated ELK-1.	1 vial
Assay Reagent	1X solution	1 vial (200 µl)
DTT	300 mM DTT	1 vial (200 µl)
Wash Buffer Concentrate (20X)	20X concentrated solution	25 ml
ELK-1 Primary Antibody	Anti- ELK-1 antibody	1 vial
HRP-conjugated Secondary Antibody	Anti-IgG HRP conjugated antibody	1 vial
Antibody Diluent Buffer	Buffer solution for diluting primary and secondary antibodies	25 ml
TMB One-Step Substrate Reagent	3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution	12 ml
Stop Solution	0.2 M sulfuric acid	8 ml

V. ADDITIONAL MATERIALS REQUIRED

1 Microplate reader capable of measuring absorbance at 450 nm.
2 Precision pipettes to deliver 1 μl to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Tubes to prepare positive or sample mixtures.

VI. REAGENT PREPARATION

1. Preparation of samples:
   Prepare nuclear extracts or whole lysates containing targeted protein ELK-1 from cell culture or tissue. We recommend using the RayBiotech Nuclear Extraction Kit (Cat#: NE-50) to isolate nuclear proteins for subsequent use in this transcription factor assay.

2. Preparation of transcription factor binding reaction system:
   Bring all reagents to room temperature (18 - 25°C) before use. Thaw the positive control and samples and keep them on ice before adding into wells. Prepare 100 μl transcription factor binding reaction system for each well with 5 x DNA Binding Buffer, Reagent, DTT, Specific Competitor DNA Probe, Non-specific Competitor DNA Probe, and Positive Control or samples containing targeted proteins. Typical examples are shown in the table below.

   Note:
   Each reaction may be prepared in a labeled microfuge tube or directly in the coated plate well. If the reaction system is prepared directly in the coated plate wells, please add the reagents sequentially as shown in the table to get the best results.

	REACTION				
COMPONENT	Positive control	Sample	Specific competitor	Non-Specific competitor	Blank
5x DNA Binding Buffer	20 μl	20 μl	20 μl	20 μl	20 μl
Assay Reagent	1.5 μl	1.5 μl	1.5 μl	1.5 μl	1.5 μl
DTT	1 μl	1 μl	1 μl	1 μl	1 μl
Specific Competitor	-	-	10 μl	-	-
Non-specific Competitor	-	-	-	10 μl	-
Control/Sample containing proteins	5 μl	* μl	* μl	* μl	-
Total volume	bring final volume to **100μl** with deionized water	bring final volume to **100μl** with deionized water	bring final volume to **100μl** with deionized water	bring final volume to **100μl** with deionized water	bring final volume to **100μl** with deionized water

* Please note that the amount of total protein containing the target protein to be used in this test can be optimized and must be determined by the investigator.

3. Preparation of primary antibody:
   Briefly spin down the ELK-1 Primary Antibody vial. Add 60 μl of Antibody Diluent Buffer into the vial to prepare a primary antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The primary antibody concentrate should then be diluted 100-fold with the Antibody Diluent Buffer and used in step 4 of Part VII Assay Procedure.

4. Preparation of secondary antibody:
   Briefly spin down the HRP-conjugated Secondary Antibody vial before use. Add 100 μl of Antibody Diluent Buffer into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should then be diluted 100-

fold with the Antibody Diluent Buffer and used in step 6 of Part VII Assay Procedure.

5. Preparation of 1x Wash Buffer:
   Dilute 25 ml of the 20x Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If the Wash Buffer Concentrate (20x) contains visible crystals, warm to room temperature and mix gently until dissolved.

   Note:
   All reagents containing protein (positive control, samples) should be kept on ice to maintain protein stability.

   If the reaction system is prepared directly in the coated plate wells, please add the reagents sequentially as shown in the table to get the best results.

   To observe the specificity of the DNA binding activity, the amount of protein used in wells of sample, specific competitor and non-specific competitor must be the same.

   A positive control should be included every time to confirm correct operation of the experiment, however it is not necessary to run the specific competitor and non-specific competitor for each sample every time.

VII. ASSAY PROCEDURE:

1. Bring the 96-well plate to room temperature (18 - 25°C) before use. If the whole plate will not be used in this assay, place remaining wells back to 2 to 8°C or -20°C. It is recommended that all positive control and samples be run at least in duplicate.

2. Add 100 μl of each prepared transcription factor binding reaction system (see Reagent Preparation step 2) including positive control, specific competitor, non-specific competitor and sample into appropriate wells. Cover wells and incubate for 2 hours at room temperature or overnight at 4°C with gentle shaking.

3. Discard the solution and wash 4 times by filling each well with 300 μl of 1x Wash Buffer (Reagent Preparation step 5) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 μl of prepared ELK-1 Primary Antibody (Reagent Preparation step 3) to each well. Incubate for 1 hour at room temperature with gentle shaking.

5. Discard the solution. Repeat the wash as in step 3.

6. Add 100 μl of prepared HRP-conjugated Secondary Antibody (see Reagent Preparation step 4) to each well. Incubate for 1 hour at room temperature with gentle shaking.

7. Discard the solution. Wash as directed in step 3.

8. Add 100 μl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

9. Add 50 μl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

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2. Add 100 μl sample to each well.
   Incubate 2 hours at room temperature or overnight at 4°C.

⇩

3. Add 100 μl prepared primary antibody to each well.
   Incubate 1 hour at room temperature.

⇩

4. Add 100 μl prepared secondary antibody.
   Incubate 1 hour at room temperature.

⇩

5. Add 100 μl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.

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6. Add 50 μl Stop Solution to each well.
   Read at 450 nm immediately.

VIII. TYPICAL DATA

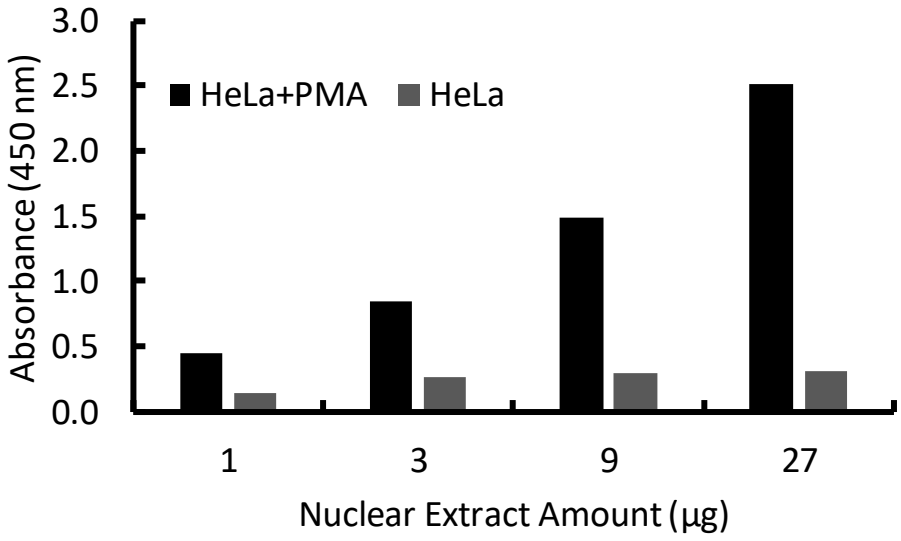

Figure 1: Transcription factor assay of ELK-1 from nuclear extracts of HeLa cells or HeLa cells treated with PMA for 3 hr. After stimulation activated ELK-1 binds with its corresponding DNA with the RayBio® TF Activity Assay Kit (cat # TFEH- ELK1).

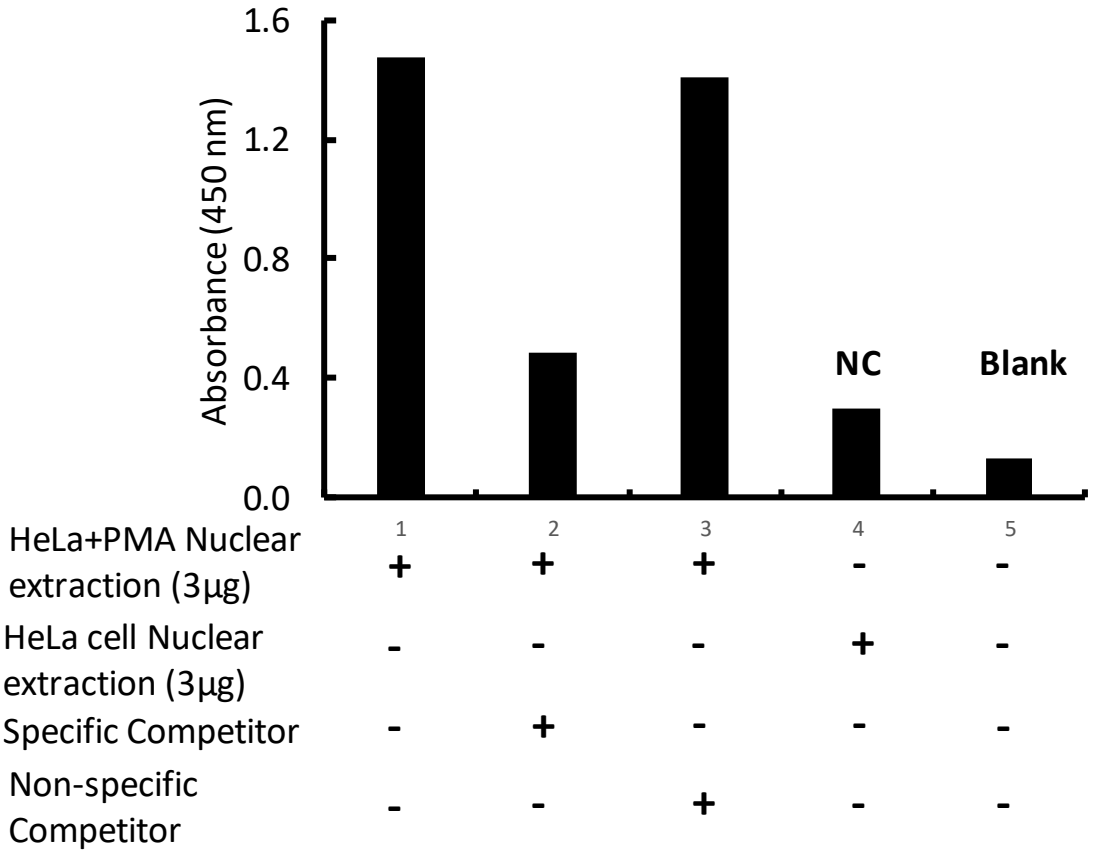

Fig. 2: Transcription factor assay of ELK-1 from nuclear extracts of HeLa cells or HeLa cells treated with PMA for 3 hr with the specific competitor or non-specific competitor. The result shows specific binding of ELK-1 to the ARE binding site detected by using the RayBio® TF Activity Assay Kit (cat # TFEH- ELK1).

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Low signal	1.Too brief incubation times	1. Ensure correction incubation time. Change incubation time in assay procedure step 2 to overnight
	2. Missed key reagent, inadequate reagent volumes or improper dilution	2. Check to ensure all reagents have been added and check pipettes to ensure correct preparation
	3. Not enough targeted protein per well	3. Check positive control wells and increase the amount of sample.
	4. Inadequate development in colorimetric assay	4. Ensure correct developing buffer and enough time used
2. Large CV	1. Inaccurate pipetting	1. Check pipettes
	2. Wells cross contamination	2. Be careful when preparing samples between wells
3. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed
	2. Contaminated wash Buffer	2. Make fresh wash buffer
	3. Incorrect antibody dilution	3. Check antibody dilutions

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