ab207195
c-Jun Transcription Factor Assay Kit (Colorimetric)

Instructions for use:

For quantitative measurement of c-Jun activation in human and mouse nuclear extracts.

This product is for research use only and is not intended for diagnostic use.

Version 1a, Last Updated 31 January 2024

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1. BACKGROUND

c-Jun Transcription Factor Assay Kit (Colorimetric) (ab207195) is a high throughput assay to quantify activator protein 1 (AP-1) c-Jun activation. This assay combines a quick ELISA format with a sensitive and specific non-radioactive assay for transcription factor activation.

A specific double stranded DNA sequence containing the TPA-responsive element (TRE) (5´ - TGAGTCA - 3´) has been immobilized onto a 96-well plate. AP1 present in the nuclear extract specifically binds to the oligonucleotide. AP1 family member c-Jun is detected by a primary antibody that recognizes an epitope of c-Jun accessible upon DNA binding. An HRP-conjugated secondary antibody provides sensitive colorimetric readout at OD 450 nm. This product detects only human and mouse c-Jun.

Key performance and benefits:

- Assay time: 3.5 hours (cell extracts preparation not included).
- Detection limit: < 1.25 µg nuclear extract/well.
- Detection range: 0.1 – 20 µg nuclear extract/well.

The activator protein-1 (AP1) transcription factors belong to a large family of structurally related transcription factors that includes ATF1-4, c-Fos, c-Jun, c-Myc and C/EBP. The members of this family, named bZIP, share a dimerization domain with a leucine zipper motif and a DNA binding domain rich in basic residues (lysines and arginines). AP1 is composed of a mixture of heterodimeric complexes of proteins derived from the Fos and Jun families including c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD. Only Jun proteins can form transcriptionally active homodimers within AP1 members, or heterodimers with CREB/ATF members, to bind the CRE element (5´ - TGACGTCA - 3´). Primarily, AP1 dimers bind to DNA on a TPA-response element (TRE) with the 5´ - TGA(C/G)TCA - 3´ sequence. Jun-Fos heterodimers form more stable complexes with TREs. These complexes display stronger transactivating activity than Jun-Jun homodimers.

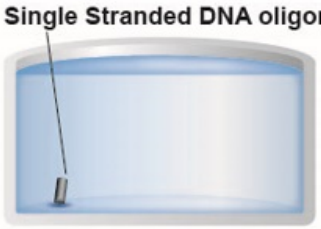
INTRODUCTION

Phosphorylation of AP1 family members by kinases is required for transactivation activity. In the case of c-Jun, the activation domain is regulated to a large extent by the JNK family of MAP kinases. JNK kinases phosphorylate c-Jun at Ser-63, resulting in the binding of c-Jun to the CBP/p300 family of transcriptional co-activators.

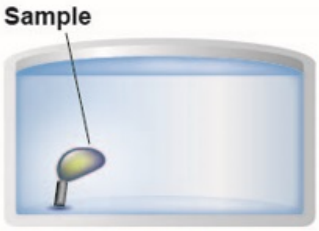
AP1 expression is induced by multiple stimuli such as serum, growth factors, phorbol esters and oncogenes. These include peptide growth factors, cytokines of the TGF beta, TNF, and interferon families, neuronal depolarization and cellular stress. Upon serum starvation of human fibroblast cells, Fos and Jun protein production can be induced for up to 4 hours by adding serum. Interestingly, serum starvation lowers basal expression of FosB and c-Fos but has no significant effect on c-Jun.

AP1 proteins play a role in the expression of many genes involved in proliferation and cell cycle progression including neuronal apoptosis, learning process, drug-induced behavioral responses, bone growth and differentiation, and embryo development. For instance, cell transformation by oncogenes that function in the growth factor signal transduction pathway, such as *ras*, *ras*F and *mek*, results in a high increase in AP1 component protein expression. Therefore, AP1-regulated genes support the invasive process observed during malignancy and metastasis.

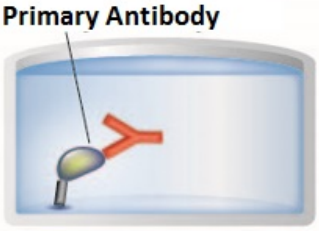
2. ASSAY SUMMARY



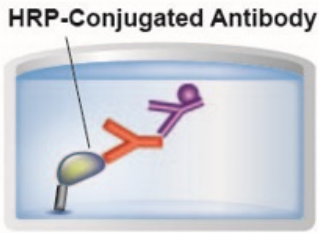
Prepare all reagents, nuclear extracts and controls as instructed. Plate is supplied pre-coated with an oligonucleotide containing the TPA-responsive element.



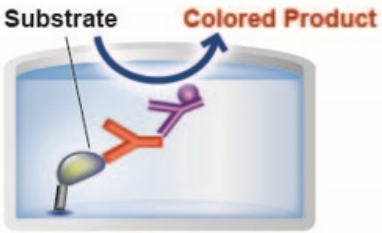
Add sample (nuclear extracts containing activated transcription factor) to appropriate wells. Incubate plate for 1 hour at RT.



Add primary antibody to wells. Incubate plate for 1 hour at RT.



Aspirate and wash each well. Add HRP-conjugated secondary antibody. Incubate plate for 1 hour at RT.



Aspirate and wash each well. Add developing solution until wells turn medium to dark blue. Add Stop Solution. Measure absorbance at OD 450 nm.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit at -20ºC (nuclear extract must be kept at -80ºC) in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt. After first use, components are stable for 6 months.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount		Storage Condition (Before Preparation)	Storage Condition (After Preparation)
	1 Plate	5 Plates		
Phospho-c-Jun antibody	22 µL	110 µL	-20°C	-20°C
Anti-rabbit HRP-conjugated IgG (0.25 µg/µL)	11 µL	55 µL	-20°C	4°C
Wild-type oligonucleotide (10 pmol/µL)	100 µL	500 µL	-20°C	-20°C
Mutated oligonucleotide (10 pmol/µL)	100 µL	500 µL	-20°C	-20°C
K-562 (TPA) nuclear extract (2.5 µg/µL)	40 µL	200 µL	-80°C	-80°C
Dithiothreitol (DTT)	100 µL	500 µL	-20°C	-20°C
Protease Inhibitor Cocktail	100 µL	500 µL	-20°C	-20°C
Poly [d(I-C)]	100 µL	500 µL	-20°C	-20°C
Lysis Buffer	10 mL	50 mL	-20°C	4°C
Binding Buffer	10 mL	50 mL	-20°C	4°C
10X Wash Buffer	22 mL	110 mL	-20°C	4°C
10X Antibody Binding Buffer	2.2 mL	11 mL	-20°C	4°C
Developing Solution	11 mL	55 mL	-20°C	4°C
Stop Solution	11 mL	55 mL	-20°C	4°C
96-well assay plate	1	5	-20°C	4°C
Plate sealer	1	5	-20°C	RT

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450nm (OD 655 nm can be used as optional reference wavelength)
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- Rocking Platform

For nuclear extract preparation:

- Hypotonic buffer (20 mM Hepes pH7.5, 5 mM NaF, 10 µM Na_2MoO_4, 0.1 mM EDTA)
- Phosphatase Inhibitors (NaF, β-glycerophosphatase, PNPP, $NaVO_3$)
- 10X PBS (0.1 M phosphate buffer pH7.5, 1.5 M NaCl, 27 mM KCl)
- NP-40

Alternatively, you can use our Nuclear Extraction Kit (ab113474) to prepare nuclear extracts.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample or control will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

Please see Quick Table for Reagent Preparation at the end of this section for a quick reference.

9.1. **Dithiothreitol (DTT, 1 M):**

Ready to use as supplied. Dilute in Lysis Buffer and Binding Buffer as described in section 9.4 and 9.5 respectively. Store at -20°C.

9.2. **Protease Inhibitor Cocktail (PIC):**

Ready to use as supplied. Dilute in Lysis Buffer as described in section 9.4. Store at -20°C.

9.3. **Poly [d(I-C)] (17 µg/µL):**

Ready to use as supplied. Dilute in Binding Buffer as described in section 9.5. Store at -20°C.

9.4. **Lysis Buffer:**

Prepare **Complete Lysis Buffer (CLB)** by adding 1 µL of 1 M DTT and 10 µL of Protease Inhibitor Cocktail to 1 mL of Lysis Buffer – see Quick Table for Reagent Preparation to see how much is required depending on number of tests. Use the CLB immediately for cell lysis. The remaining amount should be discarded if not used in the same day.

Store undiluted Lysis Buffer at 4°C.

9.5. **Binding Buffer:**

Prepare **Complete Binding Buffer (CBB)** by adding 1 µL of 1 M DTT and 10 µL of 17 µg/µL Poly [d(I-C)] to 1 mL of Binding Buffer – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Discard remaining CBB if not used in the same day. Store undiluted Binding Buffer at 4°C.

9.6. **Wash Buffer:**

Prepare **1X Wash Buffer** by making a 1/10 dilution of 10X Wash Buffer in distilled water (ddH_2O) – see Quick Table for Reagent Preparation to see how much is required depending on number of tests. Mix gently to avoid foaming.

1X Wash Buffer can be stored at 4°C for one week. ***NOTE****: Tween 20 contained in the 10X Wash Buffer may form clumps. If this happens, homogenize buffer by vortexing for 2 minutes prior to use.*

Store undiluted 10X Wash Buffer at 4°C.

9.7. **Antibody Binding Buffer:**

Prepare **1X Antibody Binding Buffer (ABB)** by making a 1/10 dilution of 10X Antibody Binding Buffer in distilled water (ddH_2O) – see Quick Table for Reagent Preparation to see how much is required depending on number of tests. Mix gently to avoid foaming.

Discard remaining 1X ABB if not used in the same day. ***NOTE:*** *BSA contained in the 10X Antibody Binding Buffer may form clumps. If this happens, homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use.*

Store undiluted 10X Antibody Binding Buffer at 4°C.

9.8. **Phospho-c-Jun Antibody (c-Jun phosphorylated at S73):**

Dilute supplied c-Jun antibody 1/500 in 1X ABB – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Aliquot and store undiluted Phospho-c-Jun antibody at -20°C. Avoid multiple freeze/thaw cycles.

9.9. **Anti-rabbit HRP-conjugated Antibody (0.25 µg/µL):**

Dilute supplied anti-rabbit HRP-conjugated antibody 1/1000 in 1X ABB – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Aliquot and store undiluted anti-rabbit HRP-conjugated antibody at 4°C. Avoid multiple freeze/thaw cycles.

Developing Solution:

Ready to use as supplied. Warm Developing Solution to room temperature 1 hour prior to use – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Store unused Developing Solution in the dark at 4°C. The Developing Solution may develop a yellow hue over time, but this does not affect product performance. A blue color present in the Developing Solution indicates that it has been contaminated and must be discarded.

9.10. **Stop Solution:**

Ready to use as supplied. Store unused Stop Solution at 4°C.

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

9.11. **K-562 (TPA stimulated) Nuclear extract (2.5 µg/µL):**

Nuclear extract was made from K-562 cells that had been stimulated with 50 ng/ml TPA for 2 hours at 37ºC. Ready to use as supplied. Extract has been optimized to be used at 5 µg/well. There is enough extract to perform 20 reactions per plate. Aliquot extract in 5 µL fractions and store at -80ºC. Avoid multiple freeze/thaw cycles.

9.12. **Control oligonucleotides (Wild-type & mutated):**

Oligonucleotides are provided to monitor the specificity of the assay.

Wild-type oligonucleotide: competes with sample nuclear extracts for the AP1 binding site.

Mutated oligonucleotide: no effect on ability of sample nuclear extracts to bind to the AP1 binding site.

Use wild-type and/or mutated oligonucleotide at 20pmol/well: dilute 2 µL appropriate oligonucleotide in 31.8 µL of CBB (section 9.5) per well used – see Quick Table for Reagent Preparation to see how much is required depending on number of tests.

Aliquot undiluted oligonucleotides and store at -20°C. Avoid multiple freeze/thaw cycles.

9.13. **96-well assay plate:**

Ready to use as supplied.

Store unused strips in the aluminium pouch at 4°C.

9.14. **Plate sealer:**

Ready to use as supplied. Store at room temperature.

Quick Table for Reagent Preparation

Reagents to prepare	Components	1 well	1 strip (8 wells)	6 strips (48 wells)	12 strips (96 wells)
Complete Lysis Buffer (CLB)	DTT	0.02 µL	0.2 µL	1.2 µL	2.4 µL
	PIC	0.23 µL	1.8 µL	10.8 µL	21.6 µL
	Lysis Buffer	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 (CBB)	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	33.4 µL	267 µL	1.602 mL	3.2 mL
	TOTAL REQUIRED	**33.8 µL**	**270 µL**	**1.62 mL**	**3.24 mL**
Oligo (wt or mutated) in CBB	Wt or mutated oligo	2 µL	16 µL	96 µL	N/A
	CBB	31.8 µL	254 µL	1.524 mL	N/A
	TOTAL REQUIRED	**33.8 µL**	**270 µL**	**1.62 mL**	**N/A**
1X Wash Buffer	ddH_2O	2.025 mL	16.2 mL	97.2 mL	194.4 mL
	10X Wash Buffer	225 µL	1.8 mL	10.8 mL	21.6 mL
	TOTAL REQUIRED	**2.25 mL**	**18 mL**	**108 mL**	**216 mL**

Reagents to prepare	Components	1 well	1 strip (8 wells)	6 strips (48 wells)	12 strips (92 wells)
1X Ab Buffer*	ddH_2O	202.5 µL	1.62 mL	9.72 mL	19.44 mL
	10x ABB	22.5 µL	180 µL	1.08 mL	2.16 mL
	TOTAL REQUIRED	**225 µL**	**1.8 mL**	**10.8 mL**	**21.6 mL**
1° Ab 1/500 Dilution	p-c-Jun Ab	0.22 µL	1.6 µL	10.8 µL	21.6 µL
	1X ABB	110 µL	900 µL	5.189 mL	10.78 mL
	TOTAL REQUIRED	**110.2 µL**	**901.6 µL**	**5.2 mL**	**10.8 mL**
2° Ab 1/1000 Dilution	HRP-conj Ab	0.11 µL	0.9 µL	5.4 µL	10.8 µL
	1X ABB	110 µL	900 µL	5.4 mL	10.8 mL
	TOTAL REQUIRED	**110.11 µL**	**900.9 µL**	**5.4 mL**	**10.8 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 preparation of buffer for diluting both primary and secondary antibodies.

10. SAMPLE PREPARATION

- We recommend using our Nuclear Extraction Kit (ab113474) to prepare nuclear extracts, as it contains all necessary buffers and will help to reduce inconsistencies in the assay that may arise from using homemade or other buffers.
- Alternatively, you can refer to the protocol below.

10.1. **Prepare reagents needed:**

10X PBS	**For 250 mL:**
0.1 M Phosphate Buffer, pH 7.5	3.55g Na_2HPO_4 + 0.61g KH_2PO_4
1.5 M NaCl	21.9 g
27 mM KCl	0.5 g

Adjust to 250 mL with ddH_2O. Prepare a 1X PBS solution dilute 10X PBS solution 1/10 in ddH_2O.

Sterilize 1X PBS pH 7.5 solution by filtering through a 0.2 µm filter. Store filter-sterilized solution at 4°C.

PIB (Phosphatase Inhibitor Buffer)	**For 10 mL:**
125 mM NaF	52 mg
250 M β-glycerophosphate	0.55 g
250 mM PNPP	1.15 g
25 mM $NaVO_3$	31 mg

Adjust to 10 mL with ddH_2O. Mix the chemical by vortexing. Incubate solution at 50°C for 5 minutes. Mix again. Store at -20°C.

HB (Hypotonic Buffer)	**For 50 mL:**
20 mM Hepes, pH 7.5	0.24 g
5 mM NaF	12 mg
10 µM Na_2MoO_4	5 µL of 0.1 M solution
0.1 mM EDTA	10 µL of 0.5 M solution

Adjust pH to 7.5 with 1 N NaOH. Adjust volume to 50 mL with ddH_2O. Sterilize by filtering through a 0.2 µm filter. Store filter-sterilized solution at 4°C.

PBS/PIB: prior to use, add 0.5 mL of PIB to 10 mL of 1X PBS.

10.2. Use cells from a confluent 100-mm dish / 75 cm^2 flask. 1 x 10^7 cells yield approximately 0.5 mg of nuclear extract.

10.3. Wash cells with 10 mL of ice-cold PBS/PIB solution.

10.4. Add 10 mL of ice-cold PBS/PIB and scrape cells off the dish with a cell scraper. Transfer cells to a pre-chilled 15 mL tube and centrifuge at 300 x *g* for 5 minutes at 4°C in a pre-chilled centrifuge. Discard supernatant.

10.5. Resuspend pellet in 1 mL of ice-cold HB buffer by gently pipetting and transfer the cells into a pre-chilled 1.5 mL tube.

10.6. Allow cells to swell on ice for 15 minutes.

10.7. Add 5 µL 10% NP-40 (0.5% final) and mix by gently pipetting.

10.8. Centrifuge homogenate for 30 seconds at 4°C in a microcentrifuge. Discard supernatant.

10.9. Resuspend nuclear pellet in 50 µL Complete Lysis Buffer (see section 9.4) and rock the tube gently on ice for 30 minutes on a shaking platform.

10.10. Centrifuge for 10 minutes at 14,000 x *g* at 4°C and save supernatant (nuclear extract).

10.11. Determine protein concentration of the extract by using a Bradford-based assay. We recommend BCA Protein Quantification Kit (ab102536).

10.12. Aliquot and store nuclear extracts at -80°C. Avoid multiple freeze/thaw cycles.

11. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommend to assay all controls and samples in duplicate.**
- **Prepare all reagents and samples as directed in the previous sections.**
- **If less than 8 strips are used, cover the unused wells with a portion of the plate sealer while performing the assay. The content of these wells is stable at RT if kept dry and can therefore be used later for a separate assay. Use strip holder for the assay.**

11.1. AP-1 c-Jun binding to its consensus sequence

11.1.1. Add 30 µL CBB containing wt or mutated oligo (Step 9.13) to the Competitive Binding Control wells.

11.1.2. Add 30 µL CBB to each of the other wells.

11.1.3. Prepare sample and control wells:

Competitive Binding control wells = 20 µL of sample diluted in CLB (use 2 – 20 µg of nuclear extract/well).

Sample wells = 20 µL of sample diluted in CLB (use 2 – 20 µg of nuclear extract/well).

Positive control wells = 2 µL of provided K-562 (TPA) nuclear extract + 18 µL CLB (5 µg nuclear extract per well).

Blank wells = 20 µL CLB only.

11.1.4. Use the provided adhesive cover to seal the plate. Incubate for 1 hour at RT with mild agitation (100 rpm on a rocking platform).

11.1.5. Wash each well 3 times with 200 µL 1X Wash Buffer. For each wash, flick plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

11.2. **Primary antibody binding**

11.2.1. Add 100 µL diluted antibody (1/500 dilution in 1X ABB) to all wells being used.

11.2.2. Cover plate and incubate for 1 hour at RT without agitation.

11.2.3. Wash each well 3 times with 200 µL 1X Wash Buffer. For each wash, flick plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

11.3. **Secondary antibody binding**

11.3.1. Add 100 µL diluted anti-rabbit HRP-antibody (1/1000 dilution in 1X ABB) to all wells being used.

11.3.2. Cover plate and incubate for 1 hour at RT without agitation.

11.3.3. During this incubation, place Developing Solution at RT.

11.3.4. Wash each well 4 times with 200 µL 1X Wash Buffer. For each wash, flick plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels

11.4. **Measurement**

11.4.1. Add 100 µL RT Developing Solution to all wells being used.

11.4.2. Incubate 2 – 10 minutes at RT protected from direct light. Optimal incubation time will vary for each experiment depending on amount of transcription factor present in the sample. Monitor the blue color development in the sample wells until it turns medium to dark blue. Do not overdevelop.

NOTE: *Positive control wells may need to overdevelop to allow adequate color development in sample wells.*

11.4.3. Add 100 µL Stop Solution to all wells being used. The solution within the wells will change from blue to yellow after adding the Stop Solution (due to presence of acid in Stop Solution).

11.4.4. Read absorbance on a spectrophotometer at OD 450 nm within 5 minutes, with a reference wavelength of OD 665 nm. Blank the plate reader according to the manufacturer's instructions using the blank wells.

12. TYPICAL DATA

TYPICAL DATA – Data provided for **demonstration purposes only**.

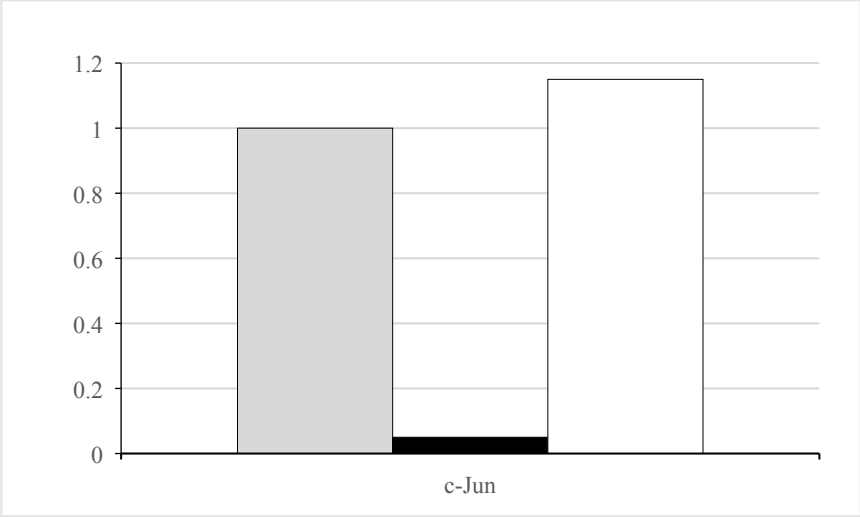


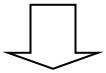
Figure 1 Nuclear extracts from K-562 cells stimulated with TPA (Gray) were assayed for activity of AP1 family member c-Jun with 5 µg/well of nuclear extract in the absence or presence of wild-type (Black) or mutated (White) consensus binding oligonucleotides. These results are provided for demonstration purposes only.

13. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

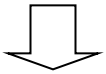
Sample binding to consensus sequence

- Add 30 µL CBB + wt/mutant oligo to Competitive Binding control wells. Add 30 µL CBB to each of the other wells used.
- Add 20 µL sample, positive control and blank to the relevant wells.
- Incubate 1 hour RT with mild agitation (100 rpm on a rocker).
- Wash each well 3 times with 200 µL 1X Washing Buffer.



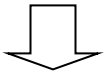
Primary antibody binding

- Add 100 µL diluted primary antibody (1/500) to each well.
- Incubate 1 hour RT with no agitation.
- Wash each well 3 times with 200 µL 1X Washing Buffer.



Secondary antibody binding

- Add 100 µL diluted HRP antibody (1/1000) to each well.
- Incubate 1 hour RT with no agitation.
- Wash each well 4 times with 200 µL 1X Washing Buffer.



Measurement

- Add 100 µL RT Development Solution to each well.
- Incubate 2 – 10 minutes RT protected from light.
- Add 100 µL Stop Solution into each well.
- Measure OD 450 nm (reference wavelength 655 nm).

14. TROUBLESHOOTING

Problem	Cause	Solution
No signal or weak signal in any well	Omission of key reagent	Check that all reagents have been added in the correct order
	Substrate or conjugate in no longer active	Test conjugate and substrate for activity
	Enzyme inhibitor present	Sodium azide will inhibit peroxidase reaction; do not add to buffers
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader
	Incorrect assay temperature	Bring reagents to room temperature
No Signal or weak signal in sample wells	Not enough nuclear extract per well	Increase amount of nuclear extract – do not exceed 50 µg/well
	c-Jun is poor activated or inactivated	Perform a time course for c-Jun activation in the studied cell line
	Extracts are not from correct species	This product detects bound phospho c- Jun (at S73) in human and mouse samples
High background in all wells	Developing time too long	Stop enzymatic reaction as soon 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
High background in sample wells	Too much nuclear extract per well	Decrease amount of nuclear extract to 1 – 2 µg/well
	Concentration of antibodies too high	Perform antibody titration to determine optimal concentration. Start with 1/2000 for 1° Ab and 1/5000 for 2° Ab. Assay sensitivity will be decreased
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

15. INTERFERENCES

These chemicals or biological materials will cause interference in this assay causing compromised results or complete failure:

- Sodium azide – it will inhibit the peroxidase reaction. Do not add to any buffer to be used in this assay.

16. NOTES

Technical Support

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