

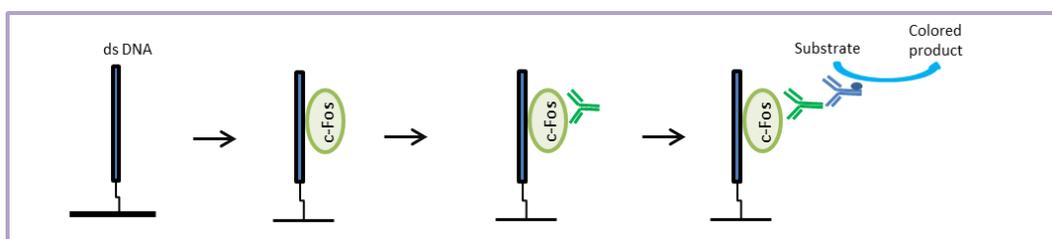
c-Fos Transcription Factor Activity Assay Kit (Colorimetric)

(Catalog # K2100-100; 100 assays; Store at Multiple Temperatures)

07/21

I. Introduction:

Activator protein-1 (aka activating protein 1, AP-1) is a critical transcription factor that plays a central role in the regulation of genes important in many cellular processes including cell proliferation, differentiation, apoptosis, survival, migration etc. AP-1 family is composed of homodimers and heterodimers of Jun, Fos, ATF or MAF protein families, which are characterized by the highly conserved dimeric basic leucine zipper (bZIP) DNA-binding domains. AP-1 protein is primarily regulated at the level of both Jun and Fos gene transcription involving MAPKs pathways and by post-translational modification via phosphorylation and dephosphorylation. AP-1 activity is induced by either physiological stimuli or environmental insults, including TPA, growth factors, polypeptide hormones, cytokines, UV irradiation, bacterial or viral infections etc. **BioVision's c-Fos Transcription Factor Activity Assay** is a 96-well plate based colorimetric assay to measure the activation of human c-Fos in nuclear extracts or cell lysates. The kit offers an easy, rapid, sensitive and non-radioactive way to detect the activation of c-Fos in samples. In this assay, double stranded DNA sequence containing the c-Fos consensus binding site is coated on the 96-well plate. Active c-Fos in the cell lysate or the nuclear extract binds to the oligonucleotides on the plate. After the addition of c-Fos primary antibody that recognizes the c-Fos-oligonucleotide complex, a HRP-conjugated secondary antibody is added followed by the addition of TMB substrate and a color signal is developed, which is measured at 450 nm.



II. Application:

- Semi-quantitative measurement of activation of human c-Fos in nuclear extracts or cell lysates.

III. Sample Types:

- Cell lysates
- Nuclear extracts

IV. Kit Contents:

Components	K2100-100	Cap Code	Part Number
Plate Coated with DNA Probes	1	--	K2100-100-1
Binding Buffer (5X)	2.2 ml	NM	K2100-100-2
DTT (100 mM)	100 µl	White	K2100-100-3
Protease Inhibitor Cocktail	20 µl	Amber	K2100-100-4
c-Fos Primary Antibody	1 ml	Green	K2100-100-5
Antibody Diluent Buffer	20 ml	WM	K2100-100-6
HRP Conjugate Stock	8 µl	Blue	K2100-100-7
Wash Buffer (10X)	27 ml	NM	K2100-100-8
Competitor Oligo	25 µl	Orange	K2100-100-9
Non-Competitor Oligo	25 µl	Red	K2100-100-10
TMB Substrate	10 ml	Amber/NM	K2100-100-11
Stop Solution	6 ml	Red	K2100-100-12
Positive Control	50 µl	Yellow	K2100-100-13
Plate Sealing Film	2	--	K2100-100-14

V. User Supplied Reagents and Equipment:

- dH₂O
- Cell lysis buffer or BioVision's Nuclear/Cytosol Fractionation Kit (BioVision Cat. No. K266).
- Multi-well spectrophotometer (ELISA plate reader)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Dounce Tissue Homogenizer (BioVision Cat. No.1998)
- Absorbent paper

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C except the Positive Control, which should be stored at -80 °C. Once the kit is opened, store the kit components as recommend below. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay

- **Plate Coated with DNA Probes:** Do not open until ready to use. Bring to room temperature (RT) before use. **After opening, immediately store the remaining unused strips at -20 °C.**
- **Binding Buffer (5X):** Store at -20 °C. Bring to RT before use. Prepare fresh Binding Buffer for the assay by adding 10 µl of 100 mM DTT and 2 µl of Proteinase Inhibitor Cocktail to 988 µl 5X Binding Buffer. Prepare enough reagents to add 100 µl/well. Use within 1 hr.
- **DTT (100 mM), Protease Inhibitor Cocktail, Competitor Oligo (20 pmol) and Non-Competitor Oligo (20 pmol):** Divide into aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles.
- **c-Fos Primary Antibody:** Divide into aliquots and store at -20 °C. Prepare c-Fos Primary Antibody working solution by adding 10 µl c-Fos Primary Antibody to 90 µl Antibody Diluent Buffer. Prepare enough reagents for the assay (100 µl/well). Keep on ice when in use.

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- **HRP Conjugate Stock:** Spin briefly before opening the vial. Prepare enough HRP Conjugate working solution to add 100 μ l/well. For example, mix 4 μ l of HRP Conjugate Stock with 7.5 ml Antibody Diluent Buffer for 70 assays. The HRP Conjugate working solution is stable at 4 $^{\circ}$ C for 2 months.
- **Wash Buffer (10X):** Bring to RT before use. Prepare 1X Wash Buffer for the assay. Prepare enough reagents for the assay. Diluted Wash Buffer can be stored for 1 month at 4 $^{\circ}$ C.
- **TMB Substrate** and **Stop Solution:** Ready to use. Store at 4 $^{\circ}$ C.
- **Positive Control (2 μ g/ μ l):** Store at -80 $^{\circ}$ C. Thaw on ice before use. Avoid repeated freeze-thaw cycles. Keep on ice when in use.

VII. AP-1/c-Fos Transcription Factor Activity Assay Protocol:

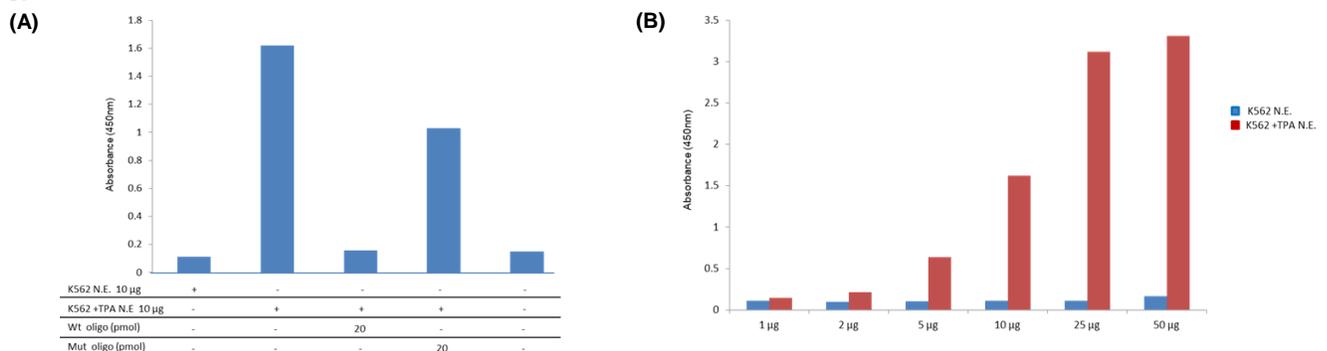
1. Sample Preparation: Cell lysate preparation: Homogenize pelleted cells ($\sim 5 \times 10^5$) with 100 μ l ice-cold cell lysis buffer using Dounce Tissue Homogenizer (BioVision Cat.No. 1998) and keep on ice for 10-15 min. Centrifuge samples at 12,000 $\times g$ and 4 $^{\circ}$ C for 15 min and collect the supernatant. **Nuclear extract preparation:** Prepare nuclear extracts using BioVision's Nuclear/Cytosol Fractionation Kit (BioVision Cat. No. K266) or any preferred method.

2. Transcription Factor Binding Reaction Mix Preparation: Prepare four different Transcription Factor Binding Reaction Mixes as shown below. **Notes:** Mix enough reagents for the number of assays to be performed. The amount of Sample used per assay should to be optimized by the researcher. A Positive Control should be included to confirm if the assay is working.

	Sample or Positive Control	Specific Competitor	Non-Specific Competitor	Background Control
Binding Buffer (5X)	20 μ l	20 μ l	20 μ l	20 μ l
Sample or Positive Control	5 μ l (10 μ g)	5 μ l (10 μ g)	5 μ l (10 μ g)	--
Competitor Oligo (20 pmole)	-	1 μ l	-	--
Non-Competitor Oligo (20 pmole)	-	-	1 μ l	--
dH ₂ O	75 μ l	74 μ l	74 μ l	80 μ l
Total Volume	100 μl	100 μl	100 μl	100 μl

- Wash each well of the **Plate Coated with DNA Probes**, 3 times with 200 μ l of 1X Wash buffer and discard the solution by decanting. Tap the inverted plate 3-5 times on a clean paper towel to remove any residual solution.
- Add 100 μ l of each **Transcription Factor Binding Reaction Mix** into appropriate wells. Cover the microtiter plate and incubate for 1 hr at RT with gentle orbital shaking (< 10 rpm).
- Decant all the reagents and wash each well 3 times as described in step 3.
- Add 100 μ l of **c-Fos Primary Antibody working solution** to each well
- Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
- Decant or aspirate all the reagents and wash each well 3 times as described in step 3.
- Add 100 μ l of **HRP Conjugate working solution** to each well.
- Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
- Decant or aspirate all the reagents and wash each well 3 times as described in step 3.
- Decant the HRP Conjugate working solution and wash each well 3 times as described in step 3.
- Add 100 μ l of **TMB Substrate** to each well. Incubate up to 30 min without shaking, protected from light. **Note:** Optimal incubation time will vary for each experiment depending on amount of transcription factor present in the sample.
- Monitor the color development in the sample wells until it turns **medium to dark blue**. **Note:** Do not overdevelop.
- Add 50 μ l **Stop Solution** to all wells and gently tap the plate to ensure thorough mixing. **Note:** The solution in the wells will change Color from blue to yellow.
- Measure the absorbance at 450 nm within 5 min at RT.

VIII. Typical Data:



Figures: Transcription factor activity assay using nuclear extracts of K562 cells were treated with 12-O-Tetradecanoylphorbol-13-acetate (TPA). Assay was performed following the assay kit protocol.

IX. Related Products:

HDAC-5 Inhibitor Screening Kit (Fluorometric) (K171)
 RelA/p65 Transcription Factor Activity Assay Kit (C) (K2093)
 c-Jun Transcription Factor Activity Assay Kit (C) (K2099)

p53 Transcription Factor Activity Assay Kit (Colorimetric) (K923)
 TFEB Transcription Factor Activity Assay Kit (Colorimetric) (K2088)

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