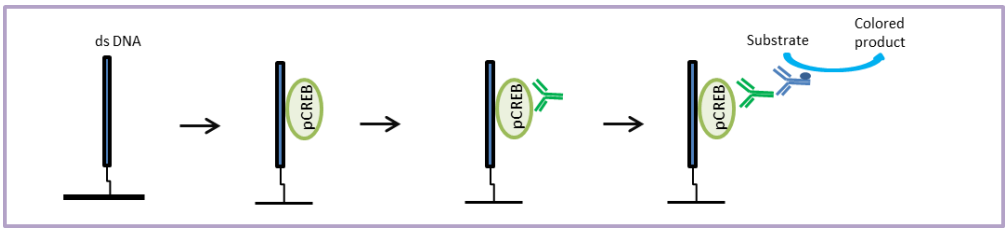
pCREB Transcription Factor Activity Assay Kit (Colorimetric)

09/21

(Catalog # K2109-100; 100 assays; Store at Multiple Temperatures)

I. Introduction:

CREB (cAMP response element-binding protein) is a phosphorylation-dependent cellular transcription factor. It is activated by phosphorylation by various serine-threonine kinases such as PKA (cAMP-dependent protein kinase A), PKC (protein kinase C) and CaMKs (calmodulin kinases) on the serine 133 residue. Once activated, pCREB interacts with the coactivator proteins and binds to the cAMP response elements (CRE) in the promoter region at 5' upstream region and increases or decreases the transcription of genes including c-fos, BDNF, tyrosine hydroxylase, numerous neuropeptides etc. CREB is involved in regulating a variety of cellular processes, including cell survival, cell proliferation, glucose homeostasis, spermatogenesis, circadian rhythm, synaptic plasticity associated with memory etc. **BioVision's pCREB Transcription Factor Activity Assay** is a 96-well plate based colorimetric assay to measure the activation of pCREB in nuclear extracts or cell lysates. The kit offers easy, rapid, sensitive and non-radioactive way to detect the activation of pCREB in samples. In this assay, double stranded DNA sequence containing the CRE is coated on the 96-well plate. Active pCREB in the cell lysate or the nuclear extract binds to the oligonucleotides on the plate. After the addition of pCREB primary antibody that recognizes the pCREB-CRE comlex, 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:

- Measure the activation of pCREB (human, mouse and rat) in nuclear extracts or cell lysates.

III. Sample Types:

- Cell lysates
- Nuclear extracts

IV. Kit Contents:

Components	K2109-100	Cap Code	Part Number
Plate Coated with DNA Probes	1	--	K2109-100-1
Binding Buffer (5X)	2.2 ml	NM	K2109-100-2
DTT (100 mM)	100 µl	White	K2109-100-3
Protease Inhibitor Cocktail	20 µl	Amber	K2109-100-4
pCREB Primary Antibody	520 µl	Green	K2109-100-5
Antibody Diluent Buffer	20 ml	WM	K2109-100-6
HRP Conjugate Stock	8 µl	Blue	K2109-100-7
Wash Buffer (10X)	27 ml	NM	K2109-100-8
Competitor Oligo	25 µl	Orange	K2109-100-9
Non-Competitor Oligo	25 µl	Red	K2109-100-10
TMB Substrate	10 ml	Amber/NM	K2109-100-11
Stop Solution	6 ml	Red	K2109-100-12
Positive Control	50 µl	Yellow	K2109-100-13
Plate Sealing Film	2	--	K2109-100-14

V. User Supplied Reagents and Equipment:

- dH_2O
- Cell lysis buffer or BioVision's Nuclear/Cytosol Fractionation Kit (BioVision Cat. No. K266)
- Multi-well spectrophotometer (ELISA 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.

- **pCREB Primary Antibody:** Divide into aliquots and store at -20 °C. Prepare pCREB Primary Antibody working solution by adding 5 µl pCREB Primary Antibody to 95 µl Antibody Diluent Buffer. Prepare enough reagents (100 µl/well). Keep on ice when in use.
- **HRP Conjugate Stock:** Spin briefly before opening the vial. Prepare enough HRP Conjugate working solution to add 100 µl/well. For example, mix 4 µl of HRP Conjugate Stock with 7.5 ml Antibody Diluent Buffer for 70 assays. The HRP Conjugate working solution is stable at 4 °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 °C.
- **TMB Substrate** and **Stop Solution:** Ready to use. Store at 4 °C.
- **Positive Control (2 µg/µl):** Store at -80 °C. Thaw on ice before use. Avoid repeated freeze-thaw cycles. Keep on ice when in use.

VII. pCREB Transcription Factor Activity Assay Protocol:

1. Sample Preparation: ***Cell lysate preparation:*** Homogenize pelleted cells (~5 x 10^5) with 100 µl 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 x *g* and 4 °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.

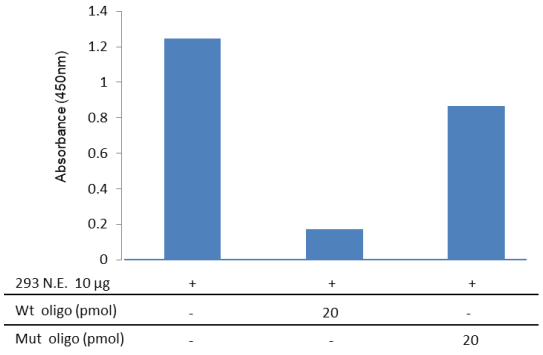
2. Transcription Factor Binding Reaction Mix Preparation: Prepare four different Transcription Factor Binding Reaction Mixes as shown below. **Notes:** Mix enough reagents for the number of assays to be performed. The amount of Sample used per assay should to be optimized by the researcher. A Positive 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 µl	20 µl	20 µl	20 µl
Sample or Positive Control	5 µl (10 µg)	5 µl (10 µg)	5 µl (10 µg)	--
Competitor Oligo (20 pmole)	-	1 µl	-	--
Non-Competitor Oligo (20 pmole)	-	-	1 µl	--
dH_2O	75 µl	74 µl	74 µl	80 µl
Total Volume	**100 µl**	**100 µl**	**100 µl**	**100 µl**

3. Wash each well of the **Plate Coated with DNA Probes,** 3 times with 200 µl 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.
4. Add 100 µl of each **Transcription Factor Binding Reaction Mix** into the appropriate wells. Cover the microtiter plate and incubate for 1 hr at RT with gentle orbital shaking (< 10 rpm).
5. Decant all the reagents and wash each well 3 times as described in step 3.
6. Add 100 µl of **pCREB Primary Antibody working solution** to each well.
7. Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
8. Decant or aspirate all the reagents and wash each well 3 times as described in step 3.
9. Add 100 µl of **HRP Conjugate working solution** to each well.
10. Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
11. Decant or aspirate all the reagents and wash each well 3 times as described in step 3.
12. Decant the HRP Conjugate working solution and wash each well 3 times as described in step 3.
13. Add 100 µl 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.
14. Monitor the color development in the sample wells until it turns **medium to dark blue**. **Note:** Do not overdevelop.
15. Add 50 µl **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.
16. Measure the absorbance at 450 nm within 5 min at RT.

VIII. Typical Data:

(A)



Figures: Transcription factor activity assay using nuclear extracts of HEK 293 cells. Assay was performed following the assay kit protocol.

IX. Related Products:

p53 Transcription Factor Activity Assay Kit (Colorimetric) (K923)
TFEB Transcription Factor Activity Assay Kit (Colorimetric) (K2088)
c-Jun Transcription Factor Activity Assay Kit (Colorimetric) (K2097)
RelA/p65 Transcription Factor Activity Assay Kit (C) (K2093)
ER Transcription Factor Activity Assay Kit (Colorimetric) (K2105)
c-Fos Transcription Factor Activity Assay Kit (Colorimetric) (K2100)

FOR RESEARCH USE ONLY! Not to be used on humans.