Datasheet for ABIN1981714

CREB1 ELISA Kit

4 Images

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Overview

Quantity:	96 tests
Target:	CREB1
Binding Specificity:	pSer133, total
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human Phospho-CREB (S133) and Total CREB ELISA Kit. This assay semi-quantitatively measures phophorylated CREB (Ser133) and Total CREB in lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human Phospho-CREB (pSer133) and total CREB.
Characteristics:	• Simultaneously measure Phosphorylated protein and pan protein in one experiment (for normalization purpose) • Screen numerous different cell lysates without performing a Western Blot analysis • Minimal hands-on time, convenient, and non-radioactive material
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer

Product Details

- Anti-Phospho Antibody
- Anti-Pan Antibody
- HRP-Conjugated Secondary Antibody
- Streptavidin-Conjugated HRP
- Assay Diluent
- TMB One-Step Substrate
- Stop Solution
- Lysis Buffer
- Positive Control Sample

Material not included:

- Distilled or deionized water
- 100 mL and 1 liter graduated cylinders
- Tubes to prepare sample dilutions
- Protease and Phosphatase inhibitors
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- Benchtop rocker or shaker
- Microplate reader capable of measuring absorbance at 450 nm

Target Details

Target:	CREB1
Alternative Name:	CREB (CREB1 Products)
Gene ID:	2345
UniProt:	P16220
Pathways:	TLR Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Thyroid Hormone Synthesis, Activation of Innate immune Response, Myometrial Relaxation and Contraction, Regulation of Cell Size, Toll-Like Receptors Cascades, G-protein mediated Events, Interaction of EGFR with phospholipase C-gamma, Positive Regulation of fat Cell Differentiation

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents and samples as instructed in the manual. 2. Add 100 µL of sample or positive control to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C.

4. Add 100 µL of prepared primary antibody to each well.
5. Incubate 1 h at RT.
6. Add 100 µL of prepared 1X HRP-Streptavidin to each well.
7. Incubate 1 h at RT.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 µL of Stop Solution to each well.
11. Read at 450 nm immediately.

Reagent Preparation:

1. Bring all reagents and samples to room temperature (18 - 25 °C) before use.

2. Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.

3. Preparation of Positive Control: Briefly spin the Positive Control vial of Item K. Add 450 µL 1x Assay Diluent (Item E) into Item K to prepare Positive Control (P-1) solution. Dissolve the powder thoroughly by a gentle mix (it can be removed by centrifuge if any precipitate in the solution is found). Pipette 300 µL 1x Assay Diluent into each tube. Use the Positive Control (P-1) solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background. (See i. Positive Control of part IX.for a typical result on page 9). Positive Control, Item K 450 µL 1x Assay Diluent P-1 P-2 P-3 P-4 0 150 µL 150µl 150 µL Phospho-CREB (S133) and Total Creb ELISA Kit Protocol 6

4. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

5. Briefly spin the detection antibody (Item C-1 or C-2) before use. Add 100 µL of 1x Assay Diluent 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 or at -80 °C for one month). The rabbit anti- CREB (S133) or mouse anti-CREB antibody concentrate should be diluted 55-fold with 1x Assay Diluent and used in step 4 of Part VII Assay Procedure.

6. Briefly spin the HRP-conjugated anti-rabbit or anti-mouse IgG (Item D- 1or D-2) before use. HRP-conjugated anti-rabbit IgG or HRP-conjugated anti-mouse IgG concentrate should be diluted 1000-fold with 1x Assay Diluent. For example: Briefly spin the vial. Add 5 µL of HRP-conjugated anti- rabbit IgG concentrate into a tube with 5.0 mL 1x Assay Diluent, pipette up and down to mix gently to prepare a 1000-fold diluted HRP- conjugated anti-rabbit IgG solution. Mix well.

7. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). Phospho-CREB (S133) and Total Creb ELISA Kit Protocol 7 VII.

Sample Preparation:

Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the

	lysis buffer. Solubilize cells at 4 x 107 cells/mL in 1x Lysis Buffer (we recommend adding protease and phosphatase inhibitors to lysis buffer prior to sample preparation). Pipette up and down to resuspend and incubate the lysates with shaking at 2 - 8°C for 30 minutes. Microcentrifuge at 13,000 rpm for 10 minutes at 2 - 8°C, and transfer the supernates into a clean test tube. Lysates should be used immediately or aliquoted and stored at -70°C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use. For the initial experiment, we recommend a serial dilution, such as 5-fold to 50-fold, for your cell lysates with Assay Diluent (Item E) before use. Note: The fold dilution of sample used depends on the abundance of phosphorylated proteins and should be determined empirically. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. Cell lysate buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). Phospho-CREB (S133) and Total Creb ELISA Kit Protocol 5
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate. 2. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to 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 1x detection antibody, anti-CREB (S133) or anti-CREB (Reagent Preparation step 5) to appropriate wells. Incubate for 1 hour at room temperature with shaking. 5. Discard the solution. Repeat the wash as in step3. 6. Add 100 µL of prepared 1x HRP-conjugated anti-rabbit IgG against anti-CREB (S133) or 1x HRP-conjugated anti-mouse IgG aganist anti- CREB (see Reagent Preparation step 6) to corresponding wells. Incubate for 1 hour at room temperature with shaking. 7. Discard the solution. Repeat the wash as in step3. 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 shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately. Phospho-CREB (S133) and Total Creb ELISA Kit Protocol 8
Calculation of Results:	ELISA data analysis: Average the duplicate readings for each sample or positive. i. Positive Control HeLa cells were treated with PMA at 37 °C for 20 min. Solubilize cells at 4 x

Application Details

	107 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI Reagent Preparation for detail. 0 0.5 1 1.5 2 O D = 4 5 0 n m P-1 P-2 P-3 P-4 P-5 Positive control dilution series Assay Diluent Phospho-CREB (S133) and Total Creb ELISA Kit Protocol 10 ii. PMA Stimulation of HeLa Cell Lines HeLa cells were treated or untreated with 250 nM PMA for 20 min. Cell lysates were analyzed using this phosphor-ELISA and Western Blot. A). ELISA 0 1 2 phospho-CREB pan-CREB O D = 4 5 0 n m Untreated PMA B). Western-Blot Analysis PMA 0 20 0 20 (Min) Anti CREB (S133) Anti pan-CREB Phospho-CREB (S133) and Total Creb ELISA Kit Protocol 11 X
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze- thaw cycles.
Storage:	-20 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20 °C. Reconstituted Positive Control (Item K) should be stored at -70 °C.
Expiry Date:	6 months

Images

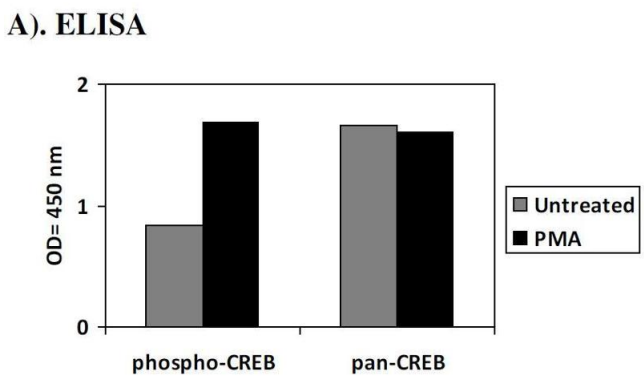

Image 1.

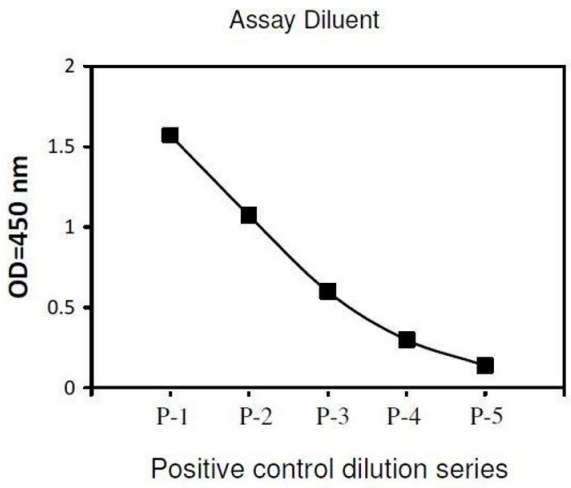

Image 2.

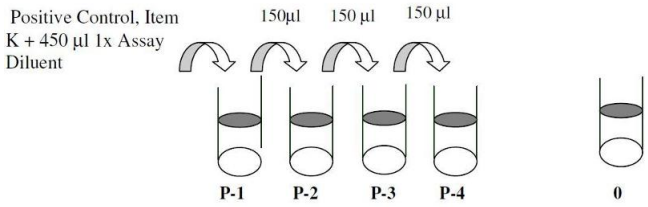

Image 3. This picture shows the preparation of the positive control.

Please check the product details page for more images. Overall 4 images are available for ABIN1981714.