



ELISA Kit
Catalog #KHO0231

**CREB
(Total)**

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

TABLE OF CONTENTS

Introduction	4
Principle of the Method.....	6
Reagents Provided.....	7
Supplies Required but Not Provided	8
Procedural Notes/Lab Quality Control.....	8
Safety	10
Directions for Washing.....	10
Procedure for Extraction of Proteins from Cells.....	11
Reagent Preparation and Storage.....	13
Reconstitution and Dilution of CREB (Total) Standard	13
Dilution of CREB (Total) Standard.....	14
Storage and Final Dilution of Anti-Rabbit IgG HRP (100X).....	15
Dilution of Wash Buffer	16
Assay Method.....	16
Typical Data	19
Limitations of the Procedure.....	20
Performance Characteristics	21
Sensitivity	21
Precision	22
Recovery.....	23
Parallelism	23
Linearity of Dilution	24
Specificity.....	25
References	26

INTRODUCTION

CREB (cAMP-Response Element-Binding protein), a protein with $M_r=43$ kDa, is a member of the large ATF/CREM/CREB transcriptional activator family. As with other members of this family, CREB contains a highly conserved leucine zipper dimerization domain and a basic DNA binding domain at its carboxyl terminus, and a unique amino terminus. CREB is ubiquitously expressed among mammalian species, and is highly conserved evolutionarily, with numerous invertebrate, plant, and yeast homologs.

CREB activates transcription in response to stimuli that elevate cytoplasmic cAMP concentrations. The series of events leading to cAMP's activation of CREB is initiated by ligand binding to certain membrane receptors, which activate adenylyl cyclase. cAMP activates a protein kinase (PKA), which translocates to the nucleus, where it phosphorylates CREB at serine 133. This phosphorylation permits CREB to recruit CREB Binding Protein (CBP), and the CREB/CBP complex in turn stimulates gene expression by interacting directly with components of the general transcriptional machinery. In addition to fostering the formation of the CREB/CBP complex, the phosphorylation of serine 133 also enhances CREB's binding to the specific DNA sequence TGACGTCA, known as the cAMP Response Element (CRE), a sequence common to the regulatory regions of genes under the control of cAMP including Bcl-2, BDNF, the immediate early gene *egr-1*, and cyclin D.

In addition to stimuli that elevate cAMP levels and activate PKA, a variety of other stimuli are observed to induce CREB serine 133 phosphorylation. These include UV irradiation, cross-linking of cell membrane proteins such as surface Ig and CD28, growth factors

including PDGF, NGF, EGF, FGF, and HGF, phorbol esters, serum feeding, and the Ca^{2+} flux that accompanies neuronal membrane depolarization. While PKA is considered to be the classical CREB kinase, other protein kinases are observed to directly phosphorylate CREB at serine 133, including the calcium/calmodulin-dependent protein kinases CaMK IV and CaMK II, Rsk -1, -2, and -3 (activated by the upstream kinase ERK1/2), and MAPKAP-K2 (activated by the upstream kinase, p38).

The Invitrogen CREB (Total) ELISA is designed to detect and quantify the level of CREB protein independent of its phosphorylation state. This assay also displays moderate cross-reactivity to a related family member, CREM. This assay is intended for the detection of CREB from lysates of human and mouse cells. This kit can be used to normalize the phosphorylated CREB content of the samples when using the CREB [pS133] ELISA Invitrogen kit (Cat. # KHO0241).

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use

PRINCIPLE OF THE METHOD

The Invitrogen CREB (Total) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for CREB (regardless of phosphorylation state) has been coated onto the wells of the microtiter strips provided. Samples, including a standard containing CREB, control specimens, and unknowns, are pipetted into these wells. During the first incubation, the CREB antigen binds to the immobilized (capture) antibody. After washing, a rabbit antibody specific for CREB is added to the wells. During the second incubation, this antibody serves as a detection antibody by binding to the immobilized CREB protein captured during the first incubation. After removal of excess detection antibody, a horseradish peroxidase-labeled Anti-Rabbit IgG (Anti-Rabbit IgG HRP) is added. This binds to the detection antibody to complete the four-member sandwich. After a third incubation and washing to remove all the excess Anti-Rabbit IgG HRP, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of CREB present in the original specimen.

REAGENTS PROVIDED

Note: Store all reagents at 2 to 8°C.

<i>Reagent</i>	<i>96 Test Kit</i>
<i>CREB (Total) Standard</i> . Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
<i>Standard Diluent Buffer</i> . Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle
<i>Antibody Coated Wells</i> . 12 x 8 Well Strips.	1 plate
<i>CREB (Total) Detection Antibody</i> . Contains 0.1% sodium azide; 11 mL per bottle.	1 bottle
<i>Anti-Rabbit IgG HRP (100X)</i> . Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
<i>HRP Diluent</i> . Contains 3.3 mM thymol; 25 mL per bottle.	1 bottle
<i>Wash Buffer Concentrate (25X)</i> . 100 mL per bottle.	1 bottle
<i>Stabilized Chromogen, Tetramethylbenzidine (TMB)</i> . 25 mL per bottle.	1 bottle
<i>Stop Solution</i> ; 25 mL per bottle.	1 bottle
<i>Plate Covers</i> , adhesive strips.	3

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Cell Extraction Buffer (see Recommended Formulation, p.11).
4. Distilled or deionized water.
5. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
6. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
7. Glass or plastic tubes for diluting and aliquoting standard.
8. Absorbent paper towels.
9. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bag.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
4. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
5. It is recommended that all standards, controls and samples be run in duplicate.
6. Extracted cell lysate samples containing CREB protein should be diluted with *Standard Diluent Buffer* at least 1:10. This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. Cover or cap all reagents when not in use.
9. **Do not mix or interchange different reagent lots from various kit lots.**
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. **Never** insert absorbent paper directly into the wells.
14. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed. If your automated washer allows, 30 second soak cycles should be programmed into the wash cycle.

PROCEDURE FOR EXTRACTION OF PROTEINS FROM CELLS

A. Recommended Formulation of Cell Extraction Buffer:

10 mM Tris, pH 7.4

100 mM NaCl

1 mM EDTA

1 mM EGTA

1 mM NaF

20 mM $\text{Na}_4\text{P}_2\text{O}_7$

2 mM Na_3VO_4

1% Triton X-100

10% glycerol

0.1% SDS

0.5% deoxycholate

1 mM PMSF (stock is 0.3 M in DMSO)

Protease inhibitor cocktail (ex., Sigma Cat. # P-2714) (reconstituted according to manufacturer's guideline). Add 250 μL per 5 mL Cell Extraction Buffer.

This buffer is stable for 2 to 3 weeks at 4°C or for up to 6 months when aliquoted (without protease inhibitors and PMSF added) and stored at -20°C. When stored frozen, the Cell Extraction Buffer should be thawed on ice. This buffer (minus protease inhibitor cocktail and PMSF) can be obtained from Invitrogen Cat. # FNN0011. **Important:** add the protease inhibitors just before using. The stability of protease inhibitor supplemented Cell Extraction Buffer is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.

B. Protocol for Cell Extraction

This protocol has been applied to several cell lines with the Cell Extraction Buffer above. Researchers may optimize the cell extraction procedures that work best in their hands.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash cells twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at -80°C and lysed at a later date).
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10 minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of CREB. For example, 10^7 HeLa cells grown in DMEM plus 10% FBS can be extracted in 1 mL of Extraction Buffer. Under these conditions, use of 1-10 μ L of the clarified cell extract diluted to a volume of 100 μ L/well in *Standard Diluent Buffer* (See **Assay Method**) is sufficient for the detection of CREB.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
6. Aliquot the clear lysate to clean microfuge tubes. These samples are ready for assay. Lysates can be stored at -80°C. Avoid multiple freeze/thaw cycles.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of CREB (Total) Standard

Note: This *CREB (Total) Standard* was prepared from purified, full length, recombinant, CREB protein expressed in *E. coli*.

1. Reconstitute *CREB (Total) Standard* with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Label as 10 ng/mL CREB. Use the standard within 1 hour of reconstitution.
2. Add 0.25 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 5, 2.5, 1.25, 0.625, 0.312, and 0.156 ng/mL of CREB.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of CREB (Total) Standard

Standard:	Add:	Into:
10 ng/mL	Prepare as described in step 1	
5 ng/mL	0.25 mL of the 10 ng/mL std.	0.25 mL of the Diluent Buffer
2.5 ng/mL	0.25 mL of the 5 ng/mL std.	0.25 mL of the Diluent Buffer
1.25 ng/mL	0.25 mL of the 2.5 ng/mL std.	0.25 mL of the Diluent Buffer
0.625 ng/mL	0.25 mL of the 1.25 ng/mL std.	0.25 mL of the Diluent Buffer
0.312 ng/mL	0.25 mL of the 0.625 ng/mL std.	0.25 mL of the Diluent Buffer
0.156 ng/mL	0.25 mL of the 0.312 ng/mL std.	0.25 mL of the Diluent Buffer
0 ng/mL	0.25 mL of the Diluent Buffer	An empty tube

Remaining reconstituted standard should be discarded or frozen at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.

C. Storage and Final Dilution of Anti-Rabbit IgG HRP (100X)

Please Note: The *Anti-Rabbit IgG HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Anti-Rabbit IgG HRP (100X)* to reach room temperature. Gently mix. Pipette *Anti-Rabbit IgG HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 μL of this 100X concentrated solution with 1 mL of *HRP Diluent* for each 8-well strip used in the assay. Label as Anti-Rabbit IgG HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Anti-Rabbit IgG HRP (100X)	Volume of Diluent
2	20 μL solution	2 mL
4	40 μL solution	4 mL
6	60 μL solution	6 mL
8	80 μL solution	8 mL
10	100 μL solution	10 mL
12	120 μL solution	12 mL

2. Return the unused *Anti-Rabbit IgG HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 100 μ L of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. Add 100 μ L of standards, samples or controls to the appropriate microtiter wells. Samples prepared in Cell Extraction Buffer must be diluted 1:10 or greater in *Standard Diluent Buffer* (for example, 10 μ L sample into 90 μ L buffer). While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal. The dilution

chosen should be optimized for each experimental system. Tap gently on side of plate to thoroughly mix. (See **REAGENT PREPARATION AND STORAGE**, Section B.)

4. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 μ L of *CREB (Total) Detection Antibody* solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover plate with *plate cover* and incubate for **1 hour at room temperature**.
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 μ L *Anti-Rabbit IgG HRP Working Solution* to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 μ L of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.

13. Incubate for **30 minutes at room temperature and in the dark.** **Please Note: Do not cover the plate with aluminum foil or metalized mylar.** The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
14. Add 100 μ L of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 μ L each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
16. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the CREB (Total) concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by dilution factor to correct for the dilution in step 3.** (Samples still producing signals higher than

the highest standard (10 ng/mL) should be further diluted in *Standard Diluent Buffer* and reanalyzed, multiplying the concentration found by the appropriate dilution factor.)

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 10 ng/mL CREB.

Standard CREB (ng/mL)	Optical Density (450 nm)
10	3.10
5	1.70
2.5	0.91
1.25	0.49
0.625	0.27
0.312	0.17
0.156	0.13
0	0.07

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 10 ng/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >10 ng/mL with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various extraction buffers has not been thoroughly investigated. The rate of degradation of native CREB in various matrices has not been investigated.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The analytical sensitivity of this assay is <0.025 ng/mL of Total CREB. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

The sensitivity of this ELISA was compared to Western blotting using known quantities of CREB. The data presented in Figure 1 show that the sensitivity of the ELISA is approximately 4x greater than that of Western blotting. The bands shown in the Western blot data were developed using rabbit anti-CREB, and an alkaline phosphatase conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

Figure 1: Detection of CREB by ELISA vs Western Blot:

Western
Blotting
(43 kDa)



ELISA:
O.D. 450 nm

0.08	0.12	0.15	0.23	0.40	0.71	1.3	2.6
------	------	------	------	------	------	-----	-----

CREB (Total)
(ng/test)

0	0.016	0.031	0.062	0.125	0.25	0.5	1
---	-------	-------	-------	-------	------	-----	---

PRECISION

1. Intra-Assay Precision

Samples of known CREB concentration were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	8.0	2.2	0.5
SD	0.1	0.07	0.03
%CV	1.4	3.4	6.8

SD = Standard Deviation

CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	8.0	2.1	0.5
SD	0.4	0.2	0.05
%CV	5.5	10.2	9.9

SD = Standard Deviation

CV = Coefficient of Variation

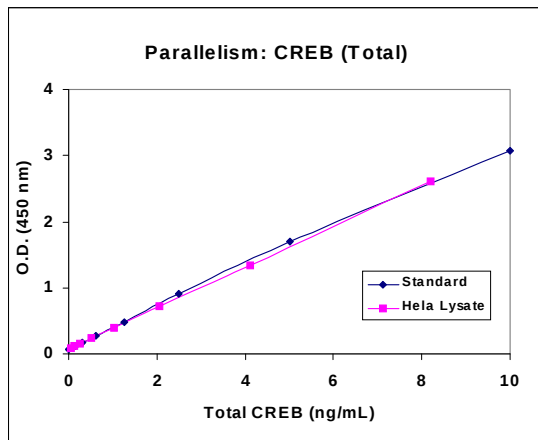
RECOVERY

To evaluate recovery, *CREB (Total) Standard* was spiked at 3 different concentrations into 10% cell extract buffer. The percent recovery was calculated as an average of 103.8%.

PARALLELISM

Natural CREB from HeLa cell lysate was serially diluted in *Standard Diluent Buffer*. The optical density of each dilution was plotted against the CREB standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects CREB content in samples.

Figure 2



LINEARITY OF DILUTION

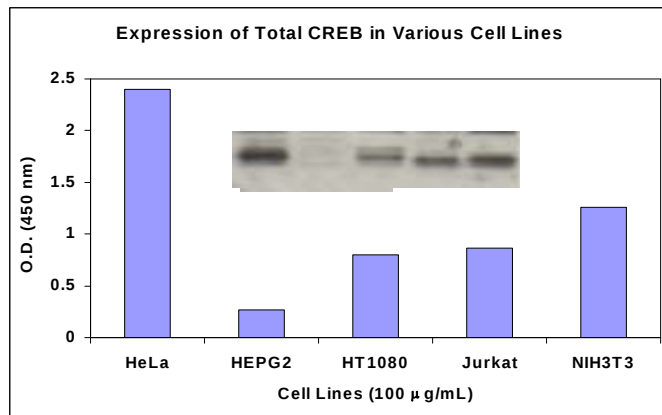
HeLa cells were grown in tissue culture medium containing 10% fetal calf serum and lysed with Cell Extraction Buffer. This lysate was diluted in *Standard Diluent Buffer* over the range of the assay and measured for CREB content. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cell Lysate		
	Measured (ng/mL)	Expected (ng/mL)	% Expected
Neat	8.2	8.2	100
1/2	3.8	4.1	93.3
1/4	1.9	2.1	93.8
1/8	0.99	1.0	96.1
1/16	0.50	0.51	97.2
1/32	0.25	0.26	96.2

SPECIFICITY

The CREB ELISA is specific for the measurement of total CREB protein and shows moderate cross-reactivity to a related family member, CREM. To determine the specificity of this ELISA kit, cell extracts from different cell lines, each at a concentration of 100 $\mu\text{g/mL}$ total protein, were analyzed. The data presented in Figure 3 show that the kit detects CREB protein in cell lysates from human HeLa, HEPG2, HT1080, and Jurkat cells, and mouse NIH3T3 cells. The levels of CREB protein detected with this ELISA kit are consistent with results obtained by Western blot analysis (insert).

Figure 3



REFERENCES

1. Impey, S. and R.H. Goodman (2001) CREB Signaling-Timing is everything. Science Signal Transduction Knowledge Environment. www.stke.org/cgi/content/full/OC_sigtrans;2001/82/pe1.
2. Shaywitz, A.J., and M.E. Greenberg (1999) CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu. Rev. Biochem. 68:821-861.
3. Xing, J., et al. (1998) Nerve growth factor activates extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways to stimulate CREB serine 133 phosphorylation. Mol. Cell. Biol. 18(4):1946-1955.
4. Maldonado, R., et al. (1996) Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. Science 273:611-612.
5. Seternes, O.M., B. Johansen, and U. Moens (1999) A dominant role for the Raf-MEK pathway in forskolin, 12-O-tetradecanoyl-phorbol acetate, and platelet derived growth factor-induced CREB (cAMP-response element-binding protein) activation, uncoupled from serine 133 phosphorylation in NIH 3T3 cells. Mol. Endocrinol. 13:1071-1081.
6. Gonzales, G.A. and M.R. Montminy (1989) Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. Cell 59:675-680.

Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		<i>In vitro</i> diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Copyright © Invitrogen Corporation. 25 February 2010

CREB (Total) Assay Summary

Incubate 100 μ L Standard or Cell Extract (>1:10)
for 2 hours at RT



↓ aspirate and wash 4x

Incubate 100 μ L of Detection Antibody
for 1 hour at RT



↓ aspirate and wash 4x

Incubate 100 μ L of HRP Anti-Rabbit Antibody
for 30 minutes at RT



↓ aspirate and wash 4x

Incubate 100 μ L of Stabilized Chromogen
for 30 minutes at RT



↓

Add 100 μ L of Stop Solution and read at 450 nm
Total time: 4 hours

