Total CREB Antibody Bead Kit
INFORMATION SHEET

Catalog #: LHO0111 **Description:** Total CREB **Lot:*** S060913

*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Intended Use

This reagent set comprises the analyte specific components for the measurement of total CREB in cell lysates/tissue homogenates. Buffer reagents needed to complete the reaction are sold separately under Catalog #LHB0002. This antibody bead kit may be multiplexed with other phospho-specific and total protein antibody bead kits available from Invitrogen, but cannot be multiplexed with the CREB [pS133] Antibody Bead Kit (Catalog #LHO0121). These reagents are intended for use in the **Luminex® 100™ or 200™ System** only. **This kit is configured for research use only and is not to be used in diagnostic procedures.**

Reagents Provided

1. Antibody Bead Concentrate (10x):

Catalog #: LM087 **Description:** CREB Beads **Lot:** S072104 **Size:** 0.25 mL-100 tests

Bead Region: 43
Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 7.5 mM sodium azide as preservative.
Storage: Store at 2 to 8°C until the expiration date indicated on the kit.

2. Detector Antibody Concentrate (10x):

Catalog #: DN087 **Description:** Total CREB Detector **Lot:** S072121 **Size:** 1 mL-100 tests

Form: 1 mL of a 10x stock of Detector Antibody Concentrate in Detector Antibody Diluent. Contains 15 mM sodium azide as preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detector Antibody.
Storage: Store at 2 to 8°C until the expiration date indicated on the kit.

3. Standard (2 vials):

Catalog #: SM020 **Description:** Total CREB Standard **Lot:** S073108 **Size:** Single use

Form: This CREB standard (lyophilized recombinant protein) is designated in ng/mL. The protein in this standard has been calibrated with the respective Invitrogen ELISA kit.
Storage: Store at 2 to 8°C. Use within 1 hour after reconstitution. Discard immediately after use.

Concentration of Reconstituted Standard:** CREB Total (10.82 ng/Ml)

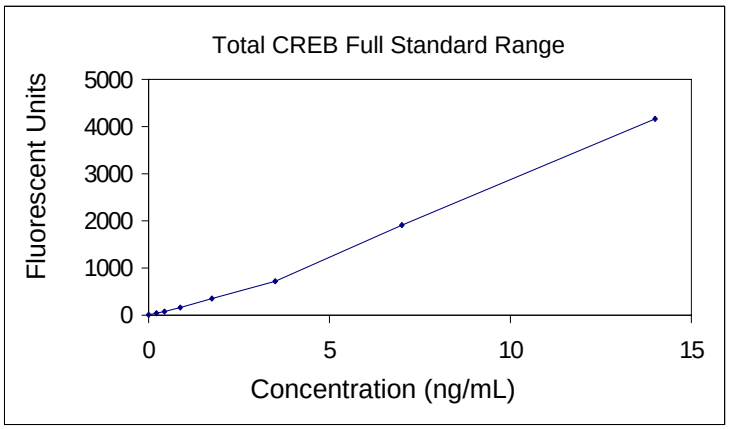
****Important note: The concentration of reconstituted standard is lot-specific. Please verify all concentration values entered in data analysis software.**

Reconstitution: Reconstitute in 1.0 mL *Assay Diluent.*

Recommended Starting Concentration for Standard Curve: Upon reconstitution, the starting concentration of standard is the value cited above. Make serial 1:2 dilutions in *Assay Diluent.* Use 100 µL per assay.

Performance Characteristics

Analytical Sensitivity: The analytical sensitivity of the total CREB assay is <0.05 ng/mL. This was determined by adding two standard deviations to the mean fluorescence units obtained when the zero standard was assayed 30 times. This sensitivity corresponds to the amount of CREB extractable from approximately 1 x 10^4 HeLa cells using NP40 Cell Lysis Buffer (formulation presented below). The assay was found to be at least twice as sensitive as Western blotting.



Representative Standard Curve

Specificity: This kit is specific for CREB, independent of its phosphorylation state, and does not display any cross-reactivity with Akt, JNK1/2, IκBα, p38, MEK1, or STAT1.

Precision:

	Intra-assay (n=16)	**Inter-assay** (n=32)
Mean (ng/mL)	5.15	5.03
SD	0.18	0.18
%CV	3.5	3.6

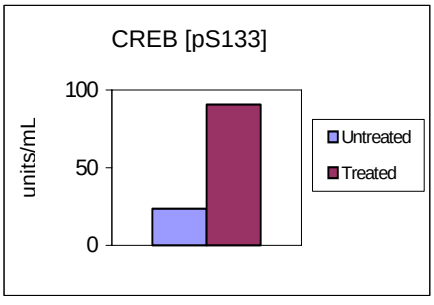
Linearity of Dilution: NP40 Cell Lysis Buffer was spiked with CREB and serially diluted in *Assay Buffer* over the range of the assay. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Recovery: To evaluate recovery, CREB was spiked at 3 different concentrations into 10% NP40 Cell Lysis Buffer. The percent recovery was calculated as an average of 101%.

Correlation to Elisa: This assay was calibrated to the mass of highly purified recombinant CREB protein expressed in *E. coli* as well as to the Invitrogen Total CREB ELISA kit (Catalog# KHO0231). The correlation coefficient was 0.97.

To further evaluate the performance of this kit, a study using forskolin was undertaken. In this study, HeLa cells grown in DMEM medium containing 10% FBS were either left untreated, or treated with 200 µM forskolin for 20 minutes at 37°C, and the levels of CREB [pS133] (Figure A) and total CREB (Figure B) were determined. This study indicated phosphorylation of CREB increased with forskolin treatment, while the level of total CREB remained approximately constant. The data presented in Figure C show the results of normalizing the level of CREB [pS133] to total CREB.

A



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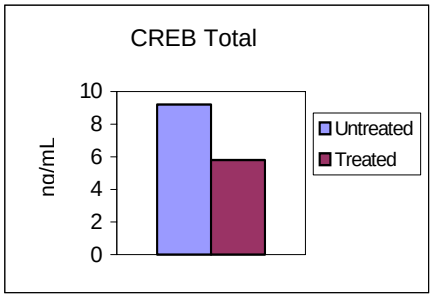

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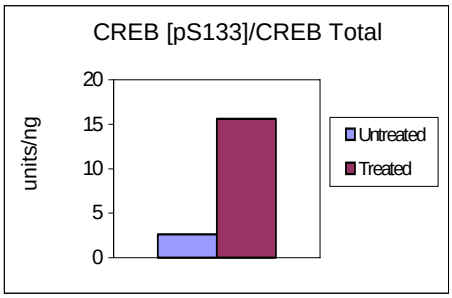
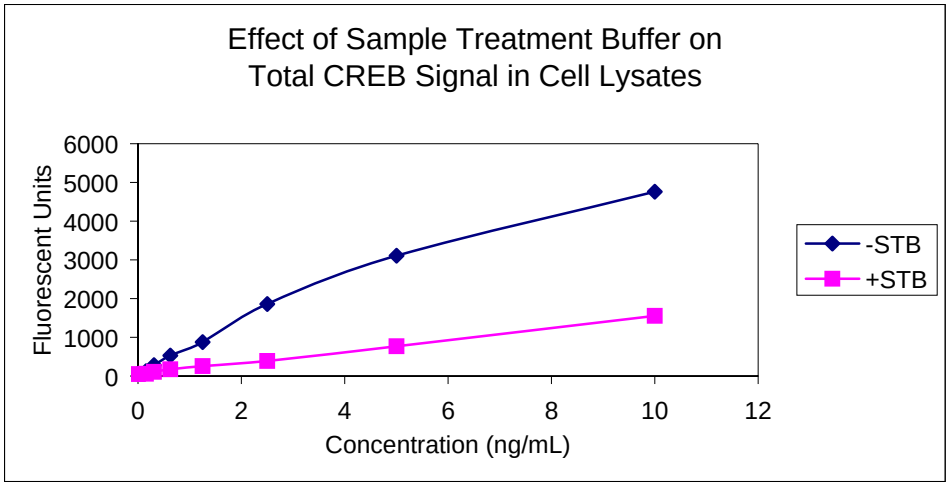


Sample Preparation:

This kit has been validated with cell lysates prepared in NP40 Cell Lysis Buffer (50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4, 1% Nonidet P40 [Roche Applied Science, Cat. # 1754599], 1 mM PMSF [stock is 0.1 M in DMSO], and protease inhibitor cocktail [Sigma Cat. # P-2714]) and diluted at least two-fold in *Assay Diluent*. To produce a lysate, incubate cells with cell lysis buffer (1-2 x 10^8 cells/mL is recommended) on ice for 30 minutes, vortexing at 10 minute intervals, then clarify the lysate by centrifugation at 13,000 rpm for 10 minutes. Cell lysates may be stored at -80°C for up to three months with one freeze/thaw cycle. Optimization of cell stimulation and cell lysis procedures may be required for each specific application.

Important Note: With some of the bead immunoassay kits available from Invitrogen, cell lysates must be pre-incubated in *Sample Treatment Buffer* to optimize signal. This sample pre-incubation step has been found to adversely impact the signal obtained with other kits. The impact of the *Sample Treatment Buffer* pre-incubation step must therefore be considered when developing multiplexed assays for the detection of multiple markers with these reagents.

The data presented below demonstrate the impact of the *Sample Treatment Buffer* pre-incubation step on the observed signal. In this study, HeLa cells were lysed in NP40 Cell Lysis Buffer at a concentration of 2 x 10^8 cells/mL cell lysis buffer. Lysates were either treated with *Sample Treatment Buffer* (+STB: lysates were diluted 1:2 in *Sample Treatment Buffer*, incubated on ice for 20 minutes, diluted 1:10 in *Assay Diluent*, and then serially diluted for measurement with the kit), or the *Sample Treatment Buffer* incubation step was omitted (-STB: lysates were diluted 1:2 in NP40 Cell Lysis Buffer, then diluted 1:10 in *Assay Diluent*, and then serially diluted for measurement with the kit).



By purchasing this Kit, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under Luminex's patent rights to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex's laser based fluorescent analytical test instrumentation marketed under the name Luminex® 100™ or 200 ™. This product is covered by one or more of the following U.S. patents: 6,046,807.