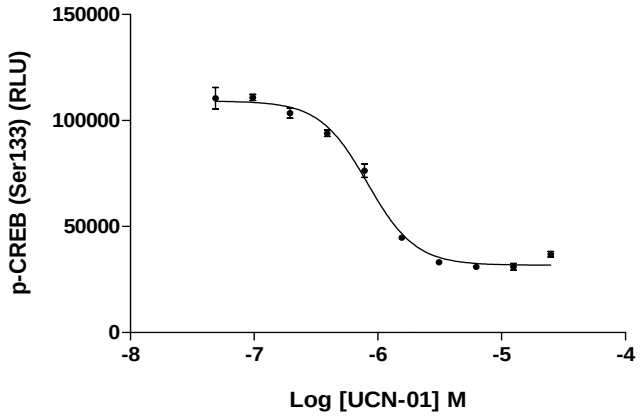
AlphaScreen® *SureFire*® phospho-CREB (Ser133) Kit

Catalog#
TGRCBS500
TGRCBS10K
TGRCBS50K

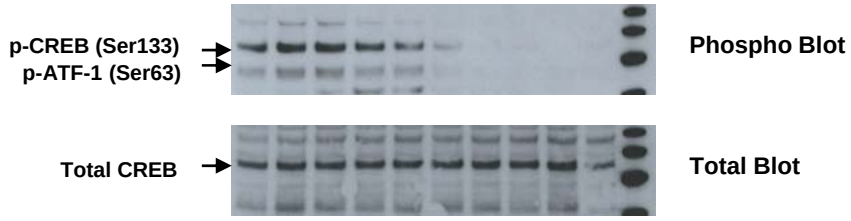
CREB is a member of the ATF/CREB transcription factor family of cyclic-AMP responsive factors that regulate several genes via binding cyclic AMP response elements in the promoter. These genes are important in regulating a broad array of cellular responses. CREB is expressed in numerous tissues, and is believed to play a key role in neuronal biology. CREB is able to selectively activate various genes through interacting with different dimerization partners. CREB activity is regulated by phosphorylation at Ser133, and can be phosphorylated at this site by several different kinases, including those involved in cAMP-mediated, MAPK-mediated, and stress-mediated signaling pathways.

A

Inhibition of EGF-mediated p-CREB (Ser133) in A431 Cells



B



Cell Handling:
A431 Cells were seeded at 150K/mL in a 96 well plate (200µl/well giving 30K cells/well) in media containing 10% FBS. Cells were incubated overnight at 37°C.

Assay Protocol:
The following day the media was removed and the cells were dose inhibited with various concentrations of **UCN-01** for 60 minutes at 37°C, then stimulated with **EGF** (200ng/mL) for 10 minutes at 37°C.

The media was removed from the wells and the cells were lysed with 50µL/well 1X Lysis Buffer with shaking for 10 minutes.

Lysates were analyzed for phospho-CREB (Ser133) and/or total CREB using either **(A)** the standard 2-plate AlphaScreen® *SureFire*® protocol (4µL lysate /384-Proxiplate well) or **(B)** Western Blot (15µL lysate/lane) with the same antibodies used for the AlphaScreen® *SureFire*® Reaction buffer.

Note: Expect phospho Ab to detect both CREB and ATF-1. Specificity for assay determined by total Ab.

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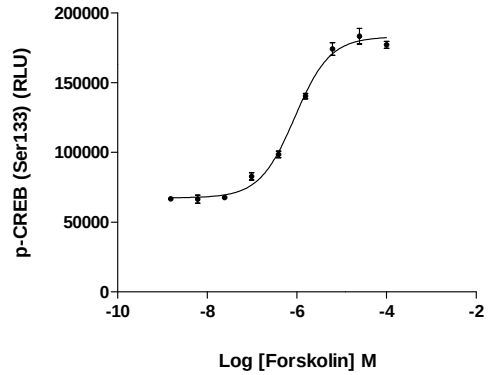
C

Forskolin-mediated p-CREB (Ser133) in A431 Cells



Agonist Dose Response Curve:

Cell Handling Protocol:
A431 Cells were seeded at 200K/mL in a 96 well plate (200µl/well giving 40K cells/well) in media containing 10% FBS. Cells were incubated overnight at 37°C.

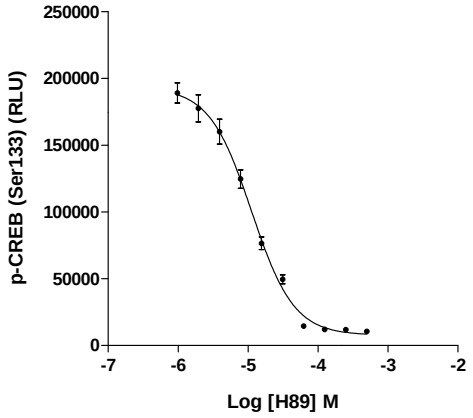
Assay Protocol:
The following day the media was removed and the cells were **serum starved** for 60 minutes at 37°C then dose stimulated with various concentrations of **Forskolin diluted in serum-free media** for 30 minutes at 37°C.

The media was removed from the wells and the cells were lysed with 50µL /well 1X Lysis buffer with shaking for 10 minutes.

Lysates (4µL) were then transferred to a 384-well Proxiplate and analyzed for phospho-CREB (Ser133) using the standard 2-plate AlphaScreen® *SureFire*® protocol.

D

Inhibition of Forskolin-mediated p-CREB (Ser133) in Hek Cells



Inhibitor Dose Response Curve:

Cell Handling Protocol:
Hek Cells were seeded at 50K/mL in a 96 well plate (200µl/well giving 10K cells/well) on Day 0 in media containing 10% FBS. Cells were incubated at 37°C.

Assay Protocol:
On Day 3 the media was removed, and the cells were treated with various concentrations of the cAMP-depedant PKA inhibitor **H89** for 60 minutes at 37°C, then stimulated with **Forskolin** (50µM) for 30 minutes at 37°C.

The media was removed from the wells and the cells were lysed with 50µL /well 1X Lysis buffer with shaking for 10 minutes.

Lysates (4µL) were then transferred to a 384-well Proxiplate and analyzed for phospho-CREB (Ser133) using the standard 2-plate AlphaScreen® *SureFire*® protocol.