Promega

Lumit™ Immunoassay Cellular System Application Note
Cellular Pathway Analysis Series

Total and Phospho-CREB (Ser 133)

Lumit™ Immunoassay Cellular System:

The Lumit™ Immunoassay Cellular System is a homogeneous bioluminescent assay that measures levels of target proteins in cell lysates when used with the appropriate primary antibody pairs (1). It combines immunodetection and NanoLuc Binary Technology (NanoBiT®) (2). In the Lumit™ Immunoassay Cellular System, NanoBiT® subunits (SmBiT and LgBiT) are conjugated to a pair of secondary antibodies against two different species (anti-rabbit, anti-mouse, or anti-goat). Seeded cells are lysed in multi-well plates using a Lumit™ compatible lysis solution and the target protein is detected by adding an antibody mix containing two primary antibodies against the target protein along with Lumit™ secondary antibodies. Binding of the primary/Lumit™ secondary antibody complexes to their corresponding epitopes brings NanoBiT® subunits into proximity to form an active NanoLuc® luciferase that makes light in proportion to the amount of the target protein (Fig. 1).

1. Hwang, B. *et al.* (2020) A homogeneous bioluminescent immunoassay approach to probe cellular signaling pathway regulation. *Commun Biol 3, 8. doi:10.1038/s42003-019-0723-9.*

2. Dixon, A. S. et al. (2016) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. *ACS Chem Biol* 11, 400-408.

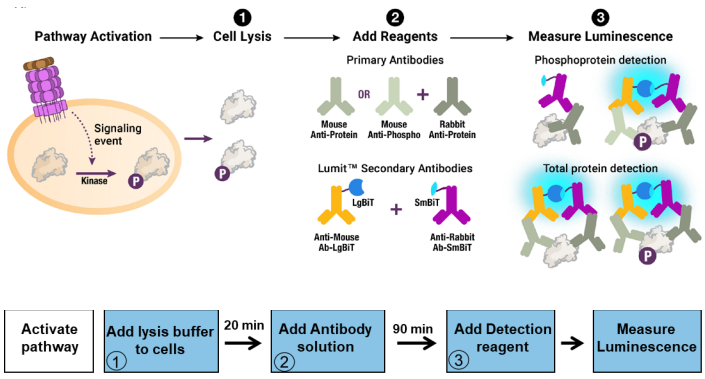

Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize non-phosphorylated epitopes on the protein (bottom panel). The luminescent signal generated is measured using a luminometer.

Total and Phospho-CREB (Ser 133) Immunoassay:

Upon activation of PKA/CREB pathway with Forskolin, CREB is phosphorylated (Fig. 2). After lysis of the cell membrane, phospho CREB (Ser 133) can be detected using the reagents in **Lumit™ Immunoassay Cellular System – Set 1** in combination with the anti-CREB antibodies described in Table 1.

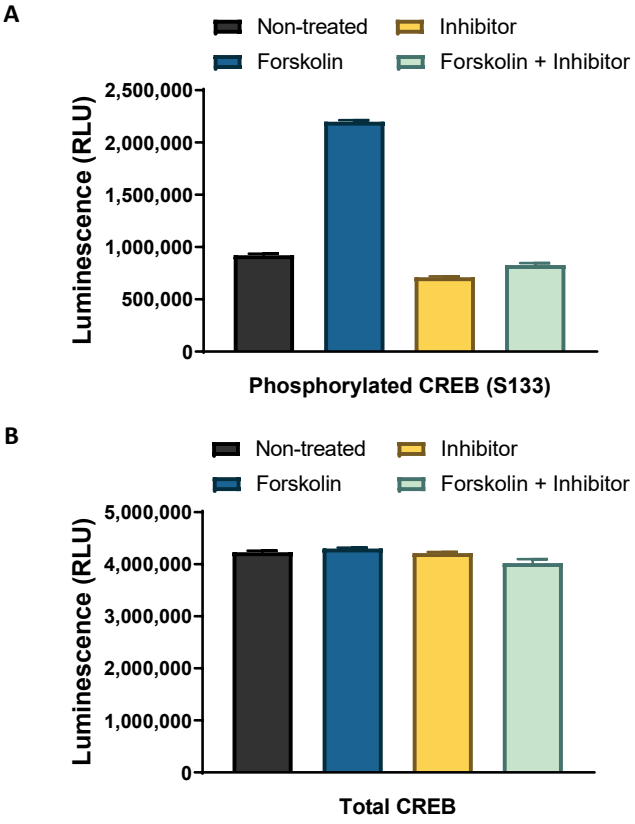

Figure 2. Detection of total and phosphorylated CREB using the Lumit™ immunoassay Cellular System – Set 1. 50,000 seeded HepG2 cells were starved overnight. The cells were then left untreated or pretreated with Staurosporine compound (0.5µM, 1hr) before they were untreated or treated with Forskolin (50µM) for 30min. Total (Panel B) and phospho-CREB (Panel A) levels were measured following Promega Technical Manual TM613 and using the primary antibody conditions described in Table 1.

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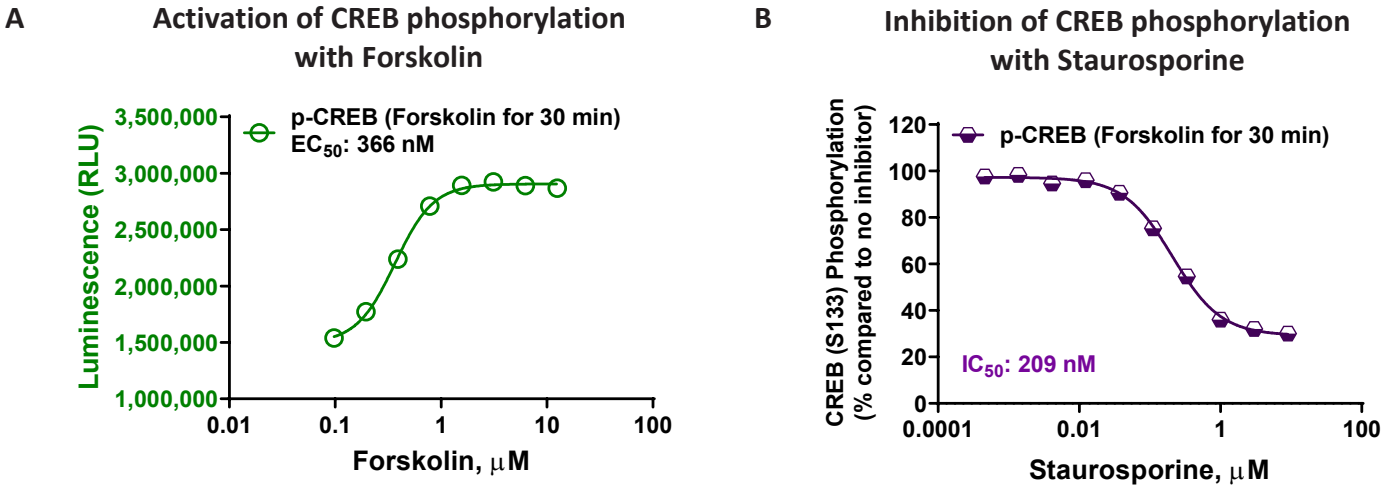

Figure 3. Activation and Deactivation of PKA pathway. (A) 50,000 seeded HepG2 cells were starved overnight. Then they were untreated or treated with various concentrations of Forskolin for 30min before phospho-CREB was measured by **Lumit™ Immunoassay Cellular System – Set 1** to determine the Forskolin EC_{50}. (B) After starvation, 50,000 seeded HepG2 cells were pretreated with various concentrations of Staurosporine for 1hr and then treated with Forskolin (30µM, 30min) before phospho-CREB was measured by **Lumit™ Immunoassay Cellular System – Set 1** to determine the potency of the inhibitor (IC_{50}).

Lumit™ Immunoassay Cellular System Short Protocol

1. Add 10µl lysis solution to 40µl cells.
2. Incubate for 20min with shaking.
3. Add 50µl Antibody mix.
4. Incubate for 60-90 min.
5. Add 25µl of Lumit™ detection reagent.
6. Shake plate for 2min.
7. Read luminescence.

This is a quick reference protocol. For more details regarding cells and reagent preparation and detailed protocols see Lumit™ Immunoassay Cellular System Technical Manual TM613 at www.promega.com/protocols.

Table 1.

Antibody*	Target	Supplier	Cat. #	Working stock (µg/ml)
p-CREB (Mouse)	Ser133	R&D Systems	MAB6906	50
CREB (Rabbit)	Total	Thermo Fisher Scientific	701120	50
CREB (Mouse)	Total	Thermo Fisher Scientific	MA1-083	50

*Antibodies from other suppliers may work as well. They may need optimization following Promega Technical Manual TM613.

Ordering Information:

Products	Size	Promega Cat. #
Lumit™ Immunoassay Cellular System – Set 1	100 assays	W1201
	1,000 assays	W1202
	10,000 assays	W1203