



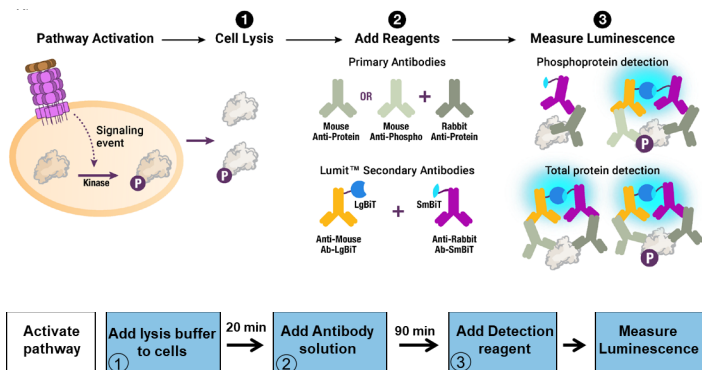
# Lumit™ Immunoassay Cellular System Application Note Cellular Pathway Analysis Series

## Phospho-c-Jun (Ser 63)

### 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).

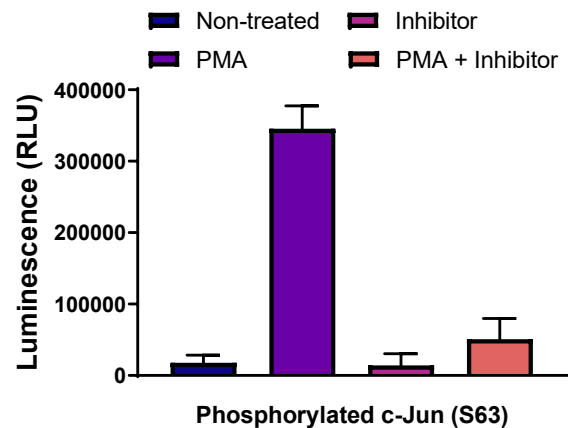
1. Hwang, B. *et al.* (2020) A homogeneous bioluminescent immunoassay approach to probe cellular signaling pathway regulation. *Commun Biol* 3, 8. doi:10.1038/s42003-019-0723-9.
2. Dixon, A. S. *et al.* (2016) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions 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.

### Phospho-c-Jun (Ser 63) immunoassay:

Upon activation of JNK pathway with PMA, c-Jun is phosphorylated (Fig. 2). After lysis of the cell membrane, phospho-c-Jun (Ser 63) can be detected using the reagents in **Lumit™ Immunoassay Cellular System – Set 1** in combination with the anti c-Jun antibodies described in Table 1.

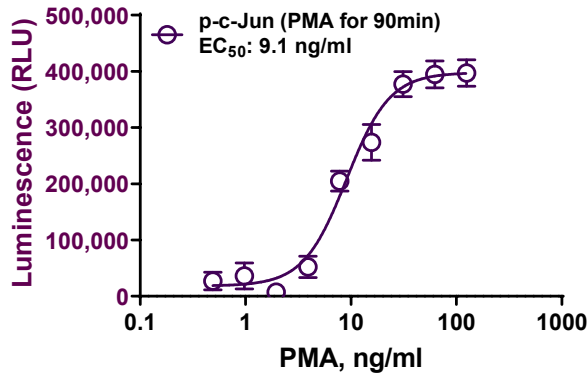


**Figure 2. Detection of phosphorylated c-Jun using the Lumit™ Immunoassay Cellular System – Set 1.** 50,000 seeded U2OS cells were serum-starved overnight. The cells were then left untreated or pretreated with SP600125 compound (50µM, 3hr) and then were untreated or treated with PMA (100ng/ml) for 90min. Phospho-c-Jun levels were measured following Promega Technical Manual TM613 and using the primary antibody conditions described in Table 1.

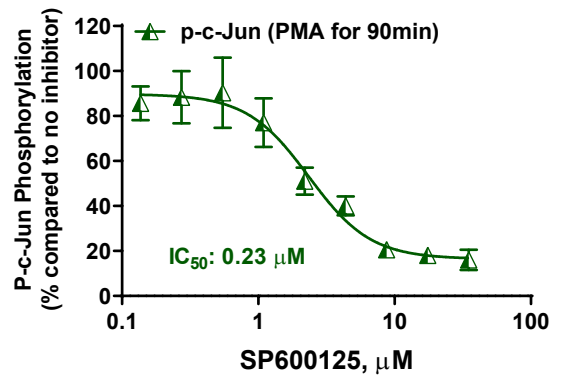
# Lumit™ Immunoassay Cellular System Application Note

## Cellular Pathway Analysis Series

### A Activation of c-Jun phosphorylation with PMA



### B Inhibition of c-Jun phosphorylation with JNK inhibitor



**Figure 3. Activation and Deactivation of JNK pathway.** 50,000 seeded U2OS cells were serum-starved overnight. (A) The cells were then untreated or treated with various concentrations of PMA for 90min before phospho-c-Jun was measured by Lumit™ Immunoassay Cellular System – Set 1 to determine the PMA EC<sub>50</sub>. (B) After starvation, the U2OS cells were pretreated with various concentrations of JNK kinase inhibitor SP600125 for 3hr and then treated with PMA (100ng/ml) for 90min before phospho-c-Jun was measured by Lumit™ Immunoassay Cellular System – Set 1 to determine the potency of the inhibitor (IC<sub>50</sub>).

### 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](http://www.promega.com/protocols).

**Table 1.**

Antibody*	Target	Supplier	Cat. #	Working stock (µg/ml)
p-c-Jun (Rabbit)	Ser 63	R&D Systems	MAB8930	50
c-Jun (Mouse)	Total	R&D Systems	MAB2670	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

