

Total and Phospho-p65 (Ser 536)

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).

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

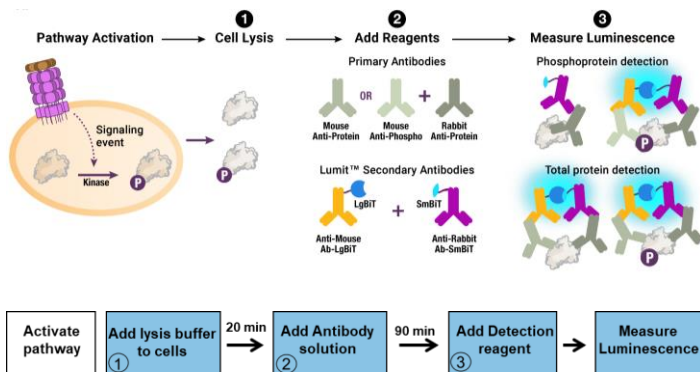


Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total and Phospho-p65 (Ser 536) Immunoassay:

Upon activation of NF-κB pathway with TNFα, p65 is phosphorylated (Fig. 2). After lysis of the cell membrane, both total and phospho-p65 (Ser 536) can be detected using the reagents in Lumit™ Immunoassay Cellular System – Set 2 in combination with the anti p65 antibodies described in Table 1.

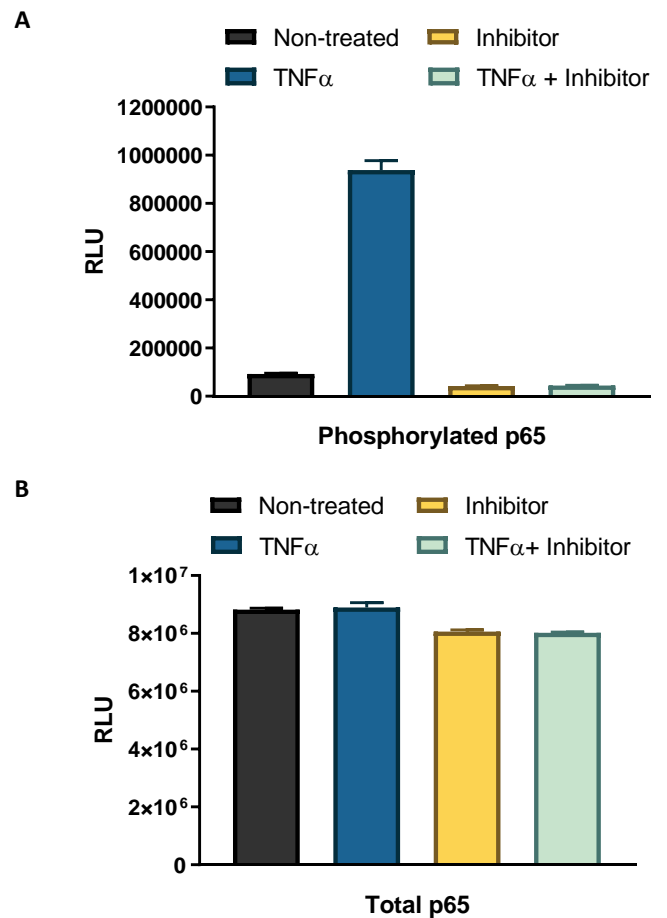


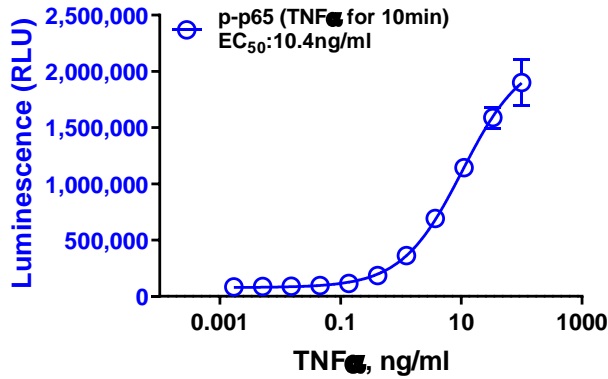
Figure 2. Detection of total and phosphorylated p65 using the Lumit™ Immunoassay Cellular System Set 2. 50,000 seeded MCF-7 cells were left untreated or pretreated with IKK16 compound (10μM, 1 hr) and then were untreated or treated with TNFα (20ng/ml) for 10min. Total and phospho-p65 levels were measured 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 p65 phosphorylation with TNF α



B Inhibition of p65 phosphorylation with IKK16

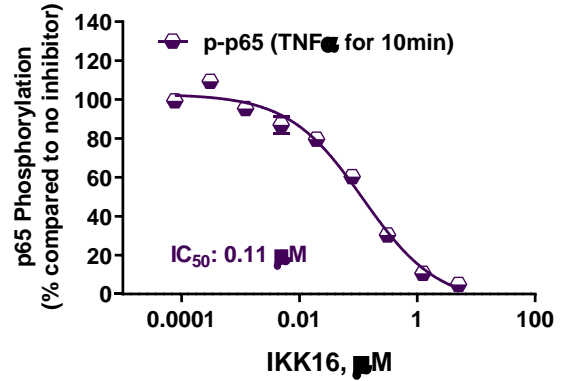


Figure 3. Activation and Deactivation of NF- κ B pathway. (A) 50,000 seeded MCF-7 were untreated or treated with various concentrations of TNF α for 10min before phospho-p65 was measured by Lumit™ Immunoassay Cellular System – Set 2 to determine the TNF α EC₅₀. (B) 50,000 seeded MCF-7 were pretreated with various concentrations of IKK16 for 1hr and then treated with TNF α (20ng/ml, 10min) before phospho-p65 was measured by Lumit™ Immunoassay Cellular System – Set 2 to determine the potency of the inhibitor (IC₅₀).

Lumit™ Immunoassay Cellular System Short Protocol

1. Add 10 μ l lysis solution to 40 μ l cells.
2. Incubate for 20min with shaking.
3. Add 50 μ l Antibody mix.
4. Incubate for 60-90 min.
5. Add 25 μ l 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 (μ g/ml)
p-p65 (Mouse)	Ser536	Cell Signaling Technology	13346	50
p65 (Rabbit)	Total	Cell Signaling Technology	8242	50
p65 (Mouse)	Total	Cell Signaling Technology	6956	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 2	100 assays	W1331
	1,000 assays	W1332
	10,000 assays	W1333