Promega

Lumit™ Immunoassay Cellular System Application Note
Cellular Pathway Analysis Series

Total and Phospho-IκBα (Ser 32)

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.

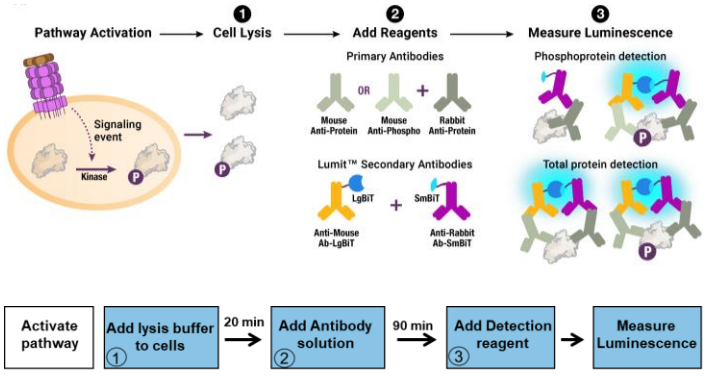

Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total and Phospho-IκBα (Ser 32) immunoassay:

Upon activation of NF-κB pathway with TNFα, IκBα is phosphorylated and then degraded (Fig. 2). After lysis of the cell membrane, both total and phospho-IκBα (Ser 32) can be detected using the reagents in **Lumit™ Immunoassay Cellular System – Set 1** in combination with the anti IκBα antibodies described in Table 1.

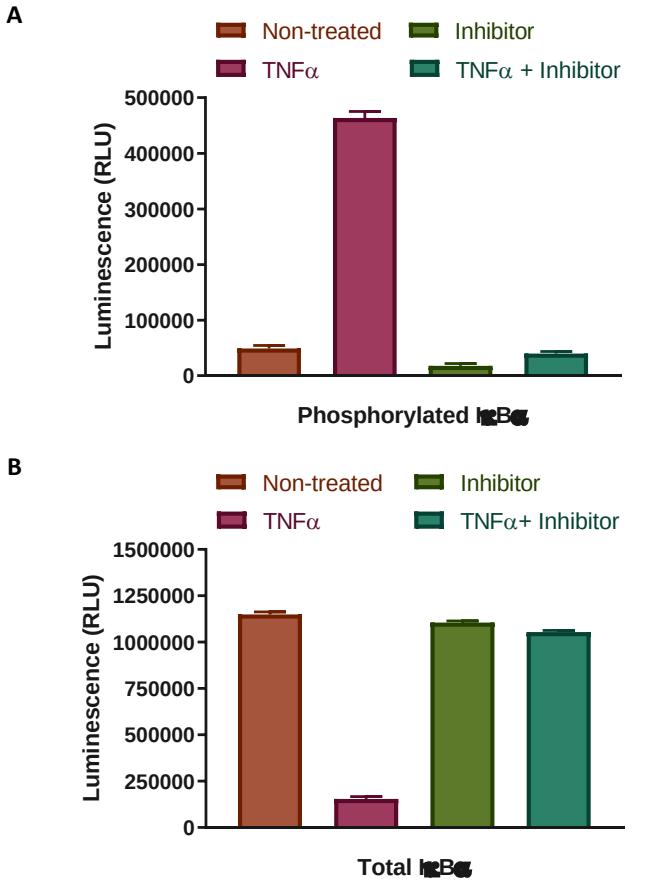

Figure 2. Detection of total and phosphorylated IκBα using the Lumit™ Immunoassay Cellular System – Set 1. 50,000 seeded MCF-7 cells were left untreated or pretreated with an IKK specific inhibitor, IKK16 compound (10µM, 1 hr) and then were untreated or treated with TNFα (20ng/ml) for 30min. For phosphorylated IκBα, cells were pretreated with MG132 (20µM, 1hr) during IKK16 treatment Total and phospho-IκBα levels were measured following Promega Technical Manual TM613 and using the primary antibody conditions described in Table 1.

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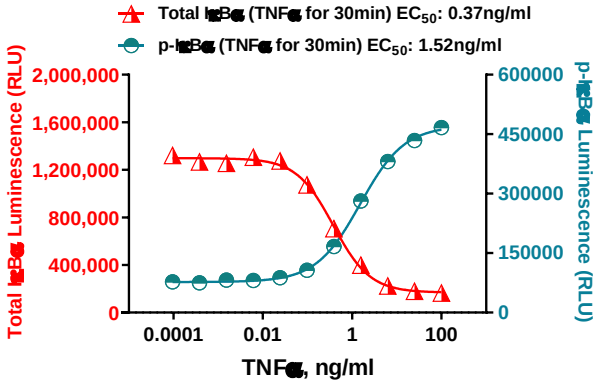
A Activation of IκBα phosphorylation and degradation with TNFα



B Inhibition of IκBα phosphorylation with IKK16

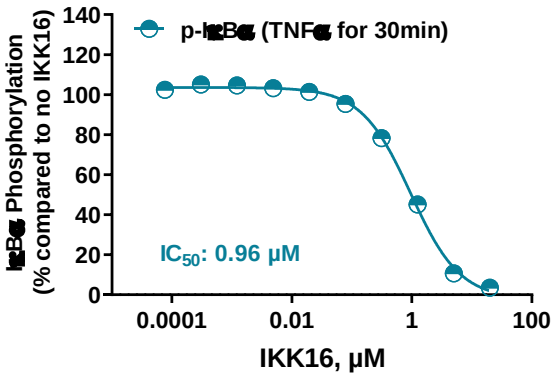


Figure 3. Activation and Deactivation of NF-κB pathway. (A) 50,000 seeded MCF-7 were untreated or treated with various concentrations of TNFα for 30min before phospho or Total IκBα were measured by **Lumit™ Immunoassay Cellular System – Set 1** to determine the TNFα EC_{50}. For p-IκBα detection, cells were pretreated with MG132 (20µM, 1hr) prior to TNFα treatment. (B) 50,000 seeded MCF-7 were pretreated with various concentrations of IKK16 for 1hr in presence of MG132 (20µM) and then treated with TNFα (20ng/ml, 30min) before p-IκBα 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-IκBα (Rabbit)	Ser32	Cell Signaling Technology	2859	50
IκBα (Mouse)	Total	Cell Signaling Technology	4814	50
IκBα (Rabbit)	Total	Cell Signaling Technology	4812	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