



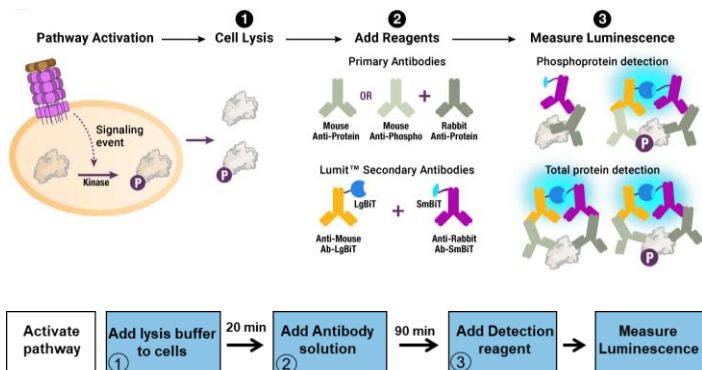
# Lumit™ Immunoassay Cellular System Application Note Cellular Pathway Analysis Series

## Phospho BTK (Tyr 223)

### 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).

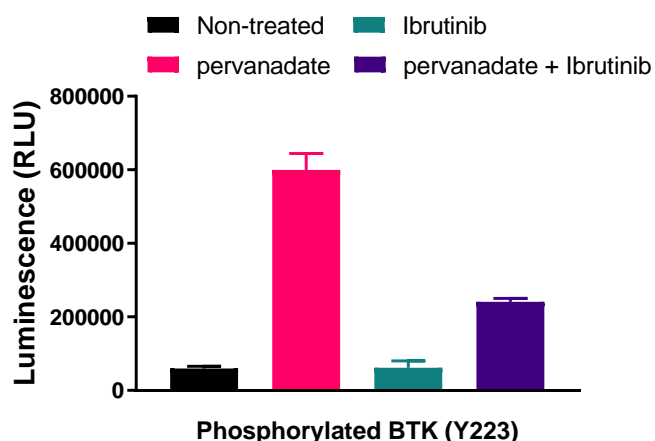
1. Hwang, B. *et al.* (2020) A homogeneous bioluminescent immunoassay approach to probe cellular signaling pathway regulation. *Commun Biol* 3, 8. doi:10.1038/s42003-019-0723-9.
2. Dixon, A. S. *et al.* (2016) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions 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 BTK (Tyr 223) Immunoassay:

Upon deactivation of BTK dephosphorylation with Pervanadate, phosphorylated BTK accumulates (Fig. 2). After lysis of the cell membrane, phospho BTK (Tyr 223) can be detected using the reagents in Lumit™ Immunoassay Cellular System – Set 1 in combination with the anti BTK antibodies described in Table 1.



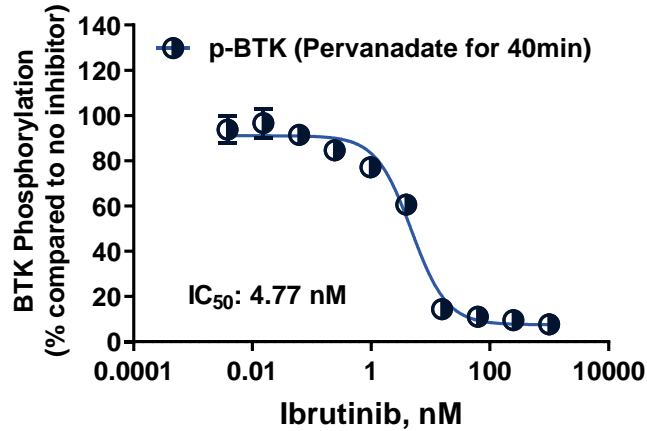
**Figure 2. Detection of phosphorylated BTK using the Lumit™ Immunoassay Cellular System – Set 1.** 180,000 seeded Ramos-RA1 cells were starved for 3 hours. The cells were then left untreated or pretreated with Ibrutinib compound (10nM, 1hr) before they were untreated or treated with Pervanadate (1mM) for 40min. Phospho BTK 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

### Inhibition of BTK phosphorylation with Ibrutinib



**Figure 3. Deactivation of BTK pathway.** After starvation for 3 hours, 180,000 seeded Ramos-RA1 cells were pretreated with various concentrations of Ibrutinib for 1hr and then treated with Pervanadate (1mM, 40min) before phospho BTK 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 50 µl 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-BTK (Rabbit)	Y223	Cell Signaling Technology	87141	50
BTK (Mouse)	Total	Cell Signaling Technology	56044	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

