

Total and Phospho-STAT3 (Tyr 705)

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.

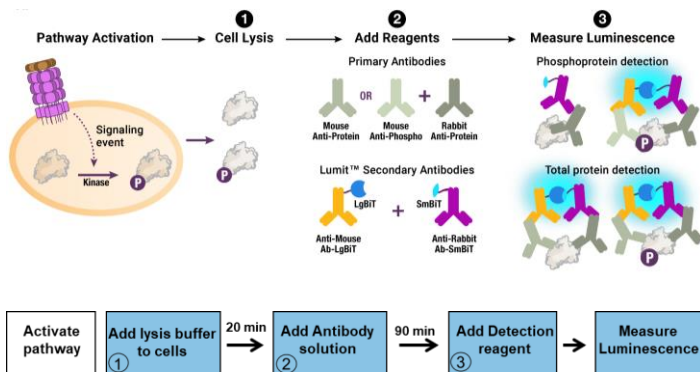


Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total and Phospho-STAT3 (Tyr 705) Immunoassay:

Upon activation of JAK/STAT pathway with Interleukin 6 (IL-6), STAT3 is phosphorylated (Fig. 2). After lysis of the cell membrane, both total and phospho-STAT3 (Tyr 705) can be detected using the reagents in Lumit™ Immunoassay Cellular System – Set 1 in combination with the anti STAT3 antibodies described in Table 1.

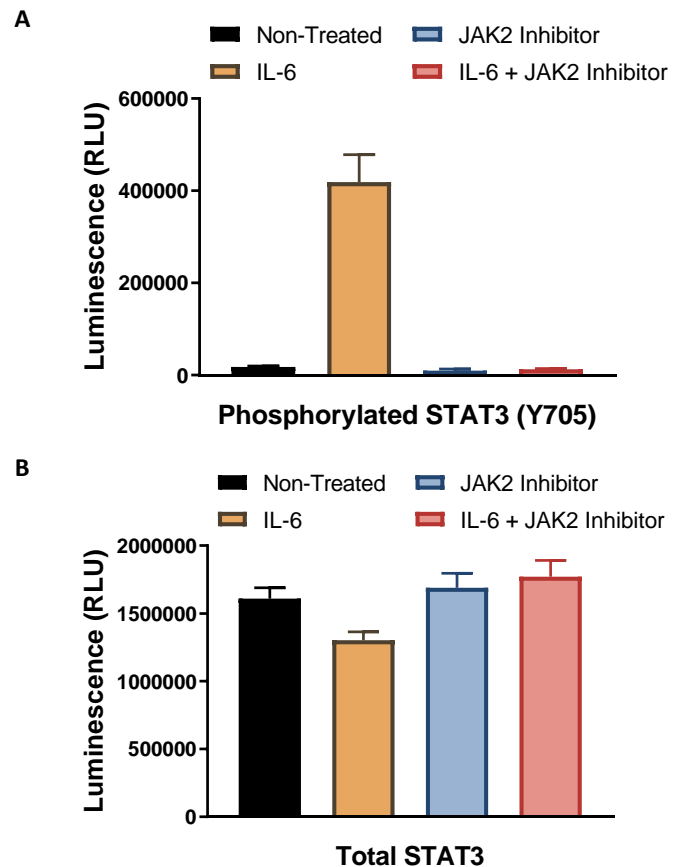
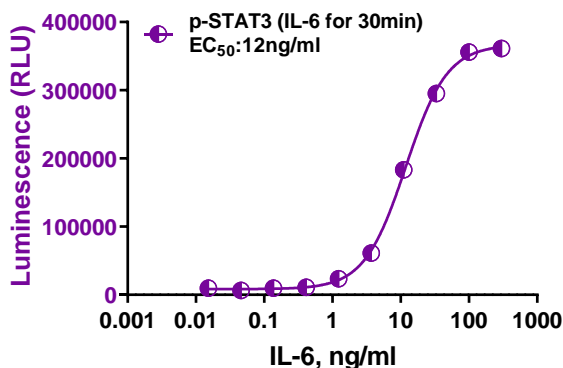


Figure 2. Detection of total and phosphorylated STAT3 using the Lumit™ immunoassay Cellular System – Set 1. 50,000 seeded A431 cells were starved overnight. The cells were then left untreated or pretreated with Ruxolitinib compound (1μM, 1hr) before they were untreated or treated with IL-6 (10ng/ml) for 30min. Total and phospho-STAT3 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 STAT3 phosphorylation with IL-6



B Inhibition of STAT3 phosphorylation with JAK inhibitors

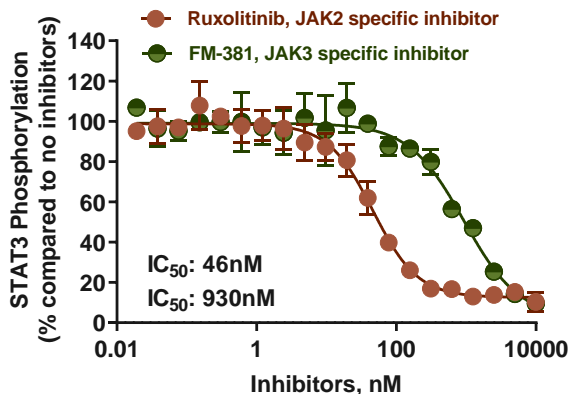


Figure 3. Activation and Deactivation of JAK/STAT pathway. (A) 50,000 seeded A431 cells were starved overnight. Then they were untreated or treated with various concentrations of IL-6 for 30min before phospho-STAT3 was measured by Lumit™ Immunoassay Cellular System – Set 1 to determine the IL-6 EC₅₀. (B) After starvation, 50,000 seeded A431 cells were pretreated with various concentrations of Ruxolitinib or FM-381 for 1hr and then treated with IL-6 (10ng/ml, 30min) before phospho-STAT3 was measured by Lumit™ Immunoassay Cellular System – Set 1 to determine the potency of the inhibitors (IC₅₀).

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-STAT3 (Rabbit)	Tyr705	Cell Signaling Technology	9145	50
STAT3 (Mouse)	Total	Cell Signaling Technology	9139	50
STAT3 (Rabbit)	Total	Cell Signaling Technology	8768	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

