

Total and Phospho-STAT1 (Tyr 701)

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.

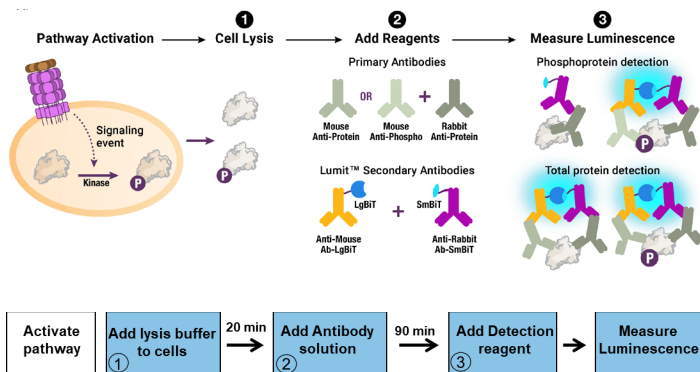


Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total and Phospho- STAT1 (Tyr701) Immunoassay:

Upon activation of JAK/STAT1 pathway with IFN γ , STAT1 is phosphorylated (Fig. 2). After lysis of the cell membrane, both total and phospho-STAT1 (Tyr 701) can be detected using the reagents in Lumit™ Immunoassay Cellular System – Set 1 in combination with the anti-STAT1 antibodies described in Table 1.

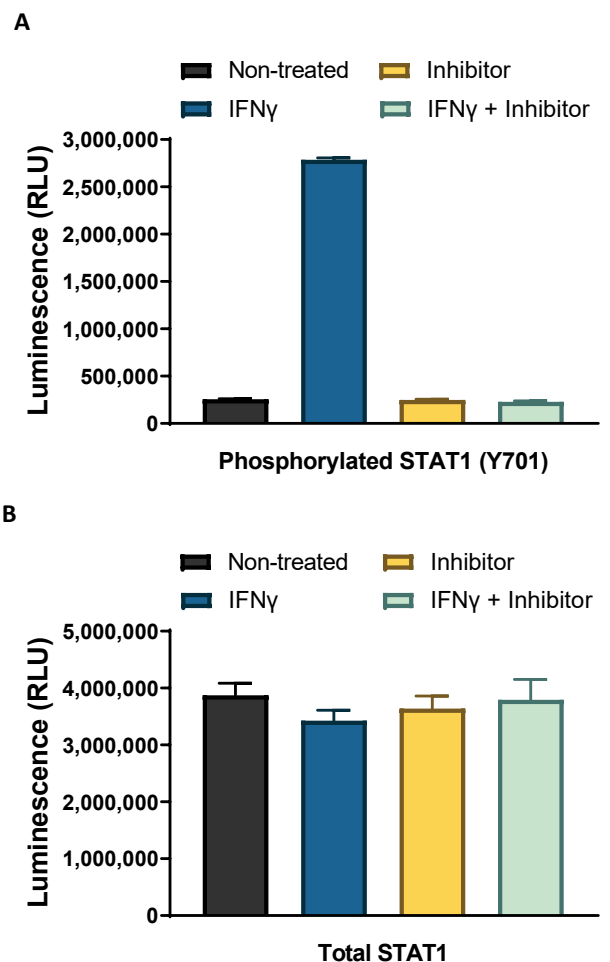
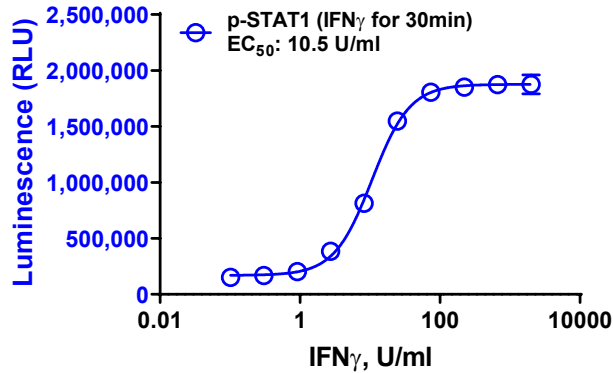


Figure 2. Detection of total and phosphorylated STAT1 using the Lumit™ Immunoassay Cellular System - Set 1. 50,000 seeded HepG2 cells were starved overnight. The cells were then left untreated or pretreated with Ruxolitinib compound (10 μ M, 1hr) before they were untreated or treated with IFN γ (1000U/ml) for 30min. Total (Panel B) and phospho-STAT1 (Panel A) 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 STAT1 phosphorylation with IFN γ



B Inhibition of STAT1 phosphorylation with Ruxolitinib

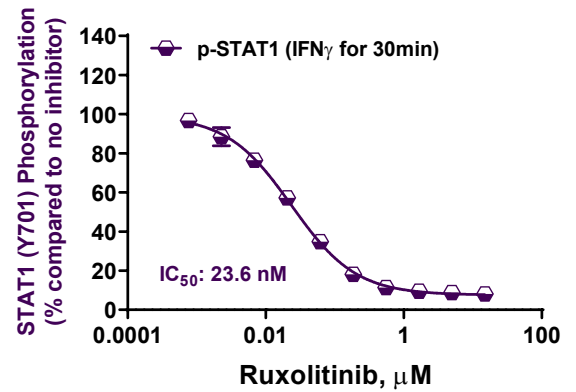


Figure 3. Activation and Deactivation of JAK/STAT1 pathway. (A) 50,000 seeded HepG2 cells were starved overnight. The cells were then untreated or treated with various concentrations of IFN γ for 30min before phospho-STAT1 was measured by Lumit™ Immunoassay Cellular System – Set 1 to determine the IFN γ EC₅₀. (B) After starvation, 50,000 seeded HepG2 cells were pretreated with various concentrations of Ruxolitinib for 1hr and then treated with IFN γ (50 U/ml, 30min) before phospho-STAT1 was measured by Lumit™ Immunoassay Cellular System – Set 1 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-STAT1 (Mouse)	Tyr701	Abcam	ab29045	50
STAT1 (Rabbit)	Total	Cell Signaling Technology	14994	50
STAT1 (Mouse)	Total	R&D Systems	MAB14901	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