Lumit™ Immunoassay Cellular System Application Note
Cellular Pathway Analysis Series

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).


Total and Phospho-STAT1 (Tyr701) Immunoassay:
Upon activation of JAK/STAT1 pathway with IFNγ, 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 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.

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 (10µM, 1hr) before they were untreated or treated with IFNγ (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.
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A  Activation of STAT1 phosphorylation with IFNγ

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 IFNγ for 30min before phospho-STAT1 was measured by Lumit™ Immunoassay Cellular System – Set 1 to determine the IFNγ EC₅₀. (B) After starvation, 50,000 seeded HepG2 cells were pretreated with various concentrations of Ruxolitinib for 1hr and then treated with IFNγ (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 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.

Table 1.

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<th>Antibody*</th>
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*Antibodies from other suppliers may work as well. They may need optimization following Promega Technical Manual TM613.

Ordering Information:

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<th>Products</th>
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