Total and Phospho-Smad1 (Ser 463/465)

**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-Smad1 (Ser463/465) Immunoassay:**

Upon activation of BMP pathway with BMP-4, Smad1 is phosphorylated (Fig. 2). After lysis of the cell membrane, both total and phospho-Smad1 (Ser463/465) can be detected using the reagents in Lumit™ Immunoassay Cellular System – Set 1 in combination with the anti-Smad1 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 Smad1 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 BMP inhibitors (10µM, 1hr) before they were untreated or treated with BMP-4 (100ng/ml) for 30min. Total (Panel B) and phospho-Smad1 (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 Smad1 phosphorylation with BMP-4

![Graph showing activation of p-Smad1 with BMP-4](image)

**Figure 3. Activation and Deactivation of BMP-4 pathway.** (A) 50,000 seeded HepG2 cells were starved overnight. The cells were then untreated or treated with various concentrations of BMP-4 for 30min before phospho-Smad1 was measured by **Lumit™ Immunoassay Cellular System – Set 1** to determine the BMP-4 EC50. (B) After starvation, 50,000 seeded HepG2 cells were pretreated with various concentrations of BMP inhibitors for 1hr and then treated with BMP-4 (30 ng/ml, 30min) before phospho-Smad1 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.

### 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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