

Total and Phospho-β-catenin (Thr 41/Ser 45)

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.

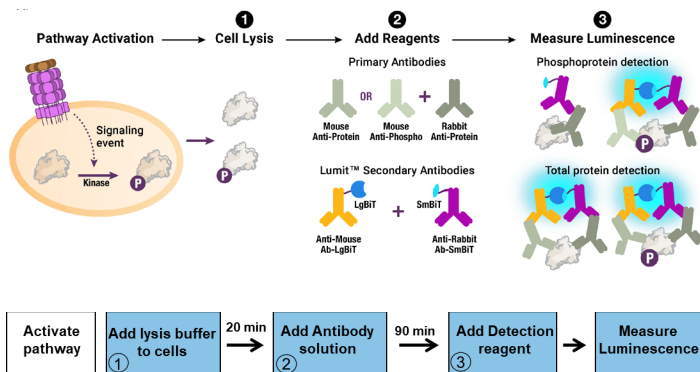


Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total and phospho-β-catenin (Thr 41/Ser 45) Immunoassay:

In the absence of Wnt ligand, β-catenin is phosphorylated and targeted to the proteasome for degradation and upon activation of WNT signaling with Wnt ligand, β-catenin is stabilized (Fig. 2) and translocates to the nucleus. After lysis of the cell membrane, total and phospho-β-catenin (Thr 41/Ser 45) can be detected using the reagents in **Lumit™ Immunoassay Cellular System – Set 2** in combination with the anti β-catenin antibodies described in Table 1.

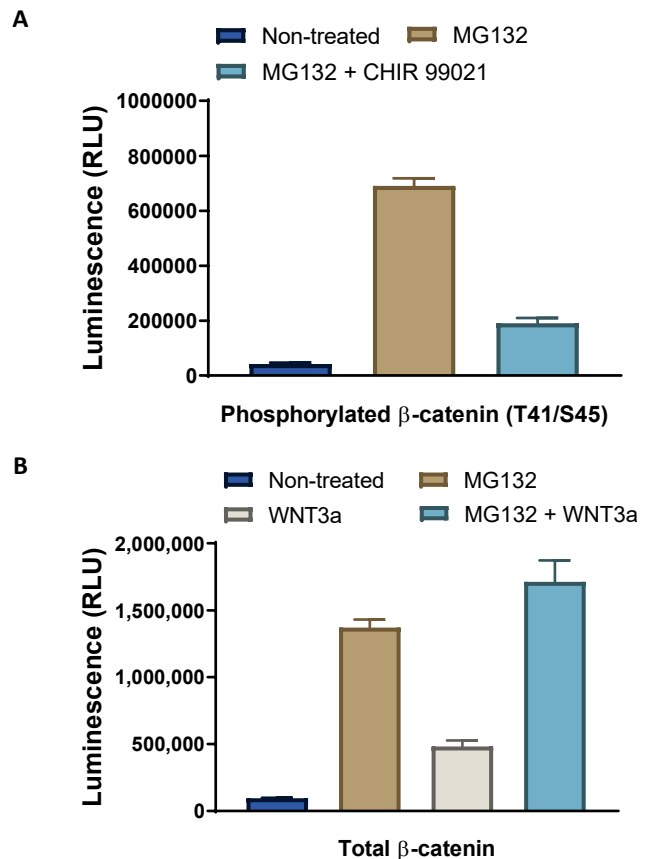
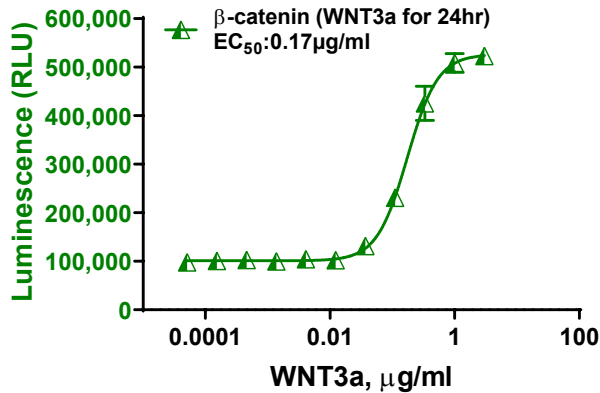


Figure 2. Detection of total and phosphorylated β-Catenin using the Lumit™ Immunoassay Cellular System – Set 2. 50,000 seeded HEK293 cells were left untreated or treated with different compounds (0.5μg/ml WNT3a, 20μM CHIR99021, and/or 20μM MG132 for 24hr). Total and phospho-β-catenin levels were measured 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 β -catenin accumulation with WNT3a



B Inhibition of β -catenin phosphorylation with GSK3- β inhibitor

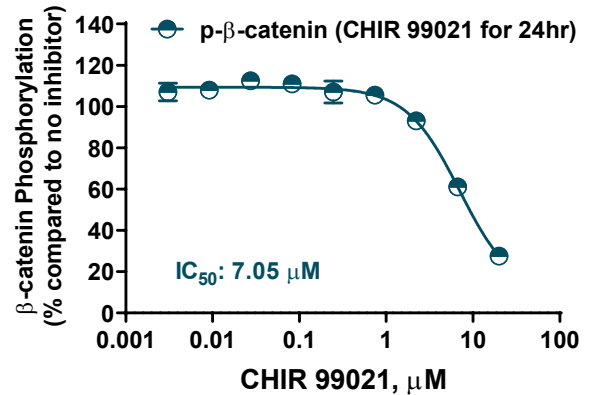


Figure 3. Activation and Deactivation of Wnt/ β -Catenin pathway. (A) 50,000 seeded HEK293 cells were untreated or treated with various concentrations of WNT3a for 24hr before Total β -catenin was measured by Lumit™ Immunoassay Cellular System – Set 2 to determine the WNT3a EC_{50} . (B) 50,000 seeded HEK293 cells were treated with various concentrations of GSK3- β inhibitor CHIR99021 in the presence of 20 μM MG132 for 24hr before phospho- β -catenin was measured by Lumit™ Immunoassay Cellular System – Set 2 to determine the potency of the inhibitor (IC_{50}).

Lumit™ Immunoassay Cellular System Short Protocol

1. Add 10 μl lysis solution to 40 μl cells.
2. Incubate for 20min with shaking.
3. Add 50 μl Antibody mix.
4. Incubate for 60-90 min.
5. Add 25 μl 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 ($\mu\text{g/ml}$)
p- β -catenin (Rabbit)	Thr 41/Ser 45	Thermo Fisher Scientific	702969	50
β -catenin (Mouse)	Total	R&D Systems	MAB13292	50
β -catenin (Rabbit)	Total	Abcam	ab196204	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 2	100 assays	W1331
	1,000 assays	W1332
	10,000 assays	W1333