Lumit™ Immunoassay Cellular System Application Note
Cellular Pathway Analysis Series

Total and Phospho-Rb (Ser 780)

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).

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.*

2. Dixon, A. S. *et al.* (2016) NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. *ACS Chem Biol* 11, 400-408.

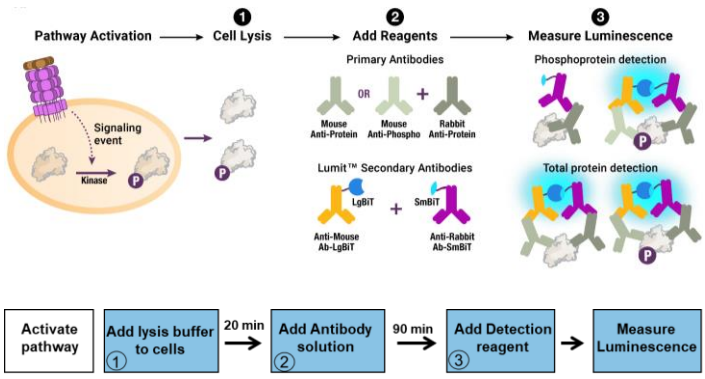

Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total and Phospho-Rb (Ser 780) Immunoassay:

Upon treatment of cells with EGF, retinoblastoma tumor suppressor protein (Rb) is phosphorylated by Cyclin-dependent kinase (Cdk) in normal and cancer cell cycles (Fig. 2). After lysis of the cell membrane, both total and phospho-Rb (Ser 780) can be detected using the reagents in **Lumit™ Immunoassay Cellular System – Set 2** in combination with the anti Rb antibodies described in Table 1.

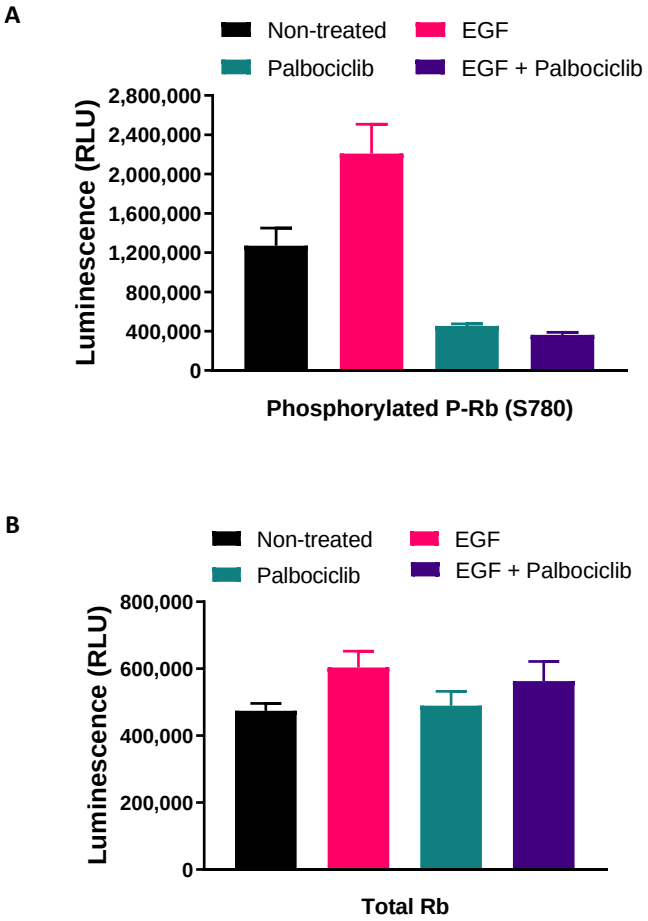

Figure 2. Detection of total and phosphorylated Rb using the Lumit™ immunoassay Cellular System – Set 2. 50,000 seeded MCF-7 cells were starved overnight. The cells were then untreated or treated with Palbociclib compound (200nM) and/or EGF (100ng/ml) for 24hrs. Total and phospho-Rb levels were measured following Promega Technical Manual TM613 and using the primary antibody conditions described in Table 1.

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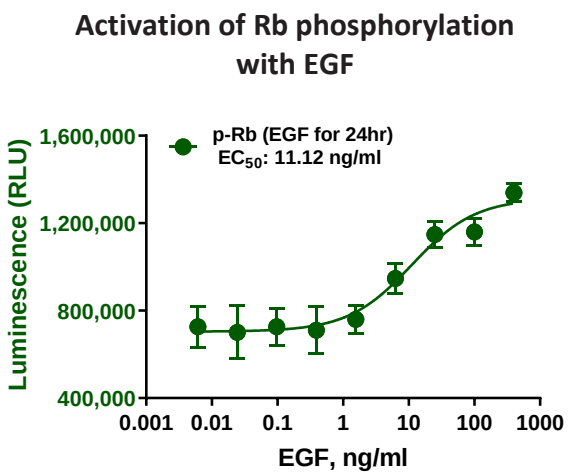
A



B

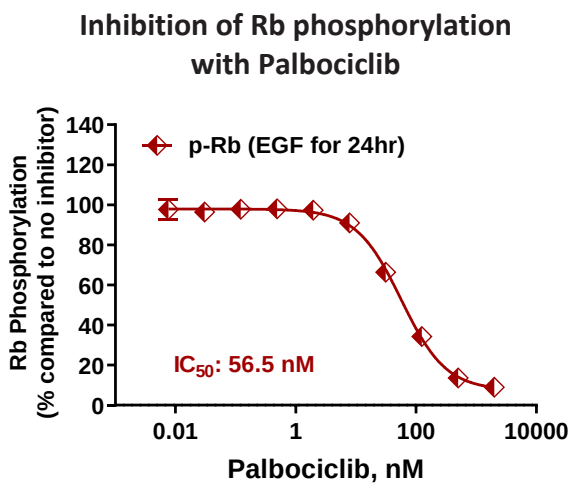


Figure 3. Activation and Deactivation of Rb/CDK pathway. 50,000 seeded MCF-7 cells were starved overnight. Then they were untreated or treated with various concentrations of EGF for 24hrs before phospho-Rb was measured by **Lumit™ Immunoassay Cellular System – Set 2** to determine the EGF EC_{50}. (B) After starvation, 50,000 seeded MCF-7 cells were treated for 24hrs with 100ng/ml EGF and various concentrations of Palbociclib before phospho-Rb 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 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-Rb (Rabbit)	Ser780	Cell Signaling Technology	8180	50
Rb (Mouse)	Total	Cell Signaling Technology	9309	50
Rb (rabbit)	Total	Thermo Fisher Scientific	MA5-32103	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