Lumit™ Immunoassay Cellular System Application Note

Cellular Pathway Analysis Series

Total and Phospho-Rb (Ser 807/811)

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 antibodies 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-Rb (Ser 807/811) 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 807/811) 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 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 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 24 hrs. Total and phospho-Rb levels were measured following Promega Technical Manual TM613 and using the primary antibody conditions described in Table 1.
A  Activation of Rb phosphorylation with EGF

![Graph showing activation of Rb phosphorylation with EGF](image)

EC$_{50}$: 5.46 ng/ml

B  Inhibition of Rb phosphorylation with Palbociclib

![Graph showing inhibition of Rb phosphorylation with Palbociclib](image)

IC$_{50}$: 78.8 nM

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.

**Table 1.**

<table>
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<tr>
<th>Antibody*</th>
<th>Target</th>
<th>Supplier</th>
<th>Cat. #</th>
<th>Working stock (µg/ml)</th>
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<tbody>
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<td>p-Rb (Rabbit)</td>
<td>Ser807/811</td>
<td>Cell Signaling Technology</td>
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<tr>
<td>Rb (Mouse)</td>
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<td>Cell Signaling Technology</td>
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<td>50</td>
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<tr>
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<td>Thermo Fisher Scientific</td>
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*Antibodies from other suppliers may work as well. They may need optimization following Technical manual TM613.

**Ordering Information:**

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<th>Products</th>
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<tr>
<td></td>
<td>1,000 assays</td>
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