Lumit™ Immunoassay Cellular System Application Note
Cellular Pathway Analysis Series

Total Estrogen Receptor (ER)

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.

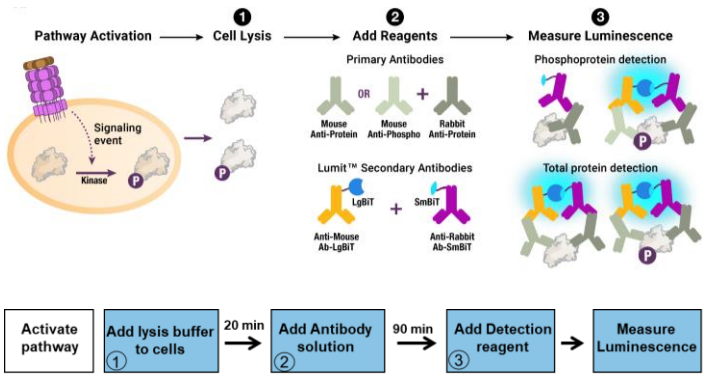

Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phospho-specific antibody, the luminescence reflects the level of the target protein phosphorylation (top panel). To detect total protein level, the same concept is used except both primary antibodies recognize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

Total Estrogen Receptor (ER) Immunoassay:

Upon treatment of cells with small molecule degrader Fulvestrant, ER is degraded (Fig. 2). After lysis of the cell membrane, total ER can be detected using the reagents in **Lumit™ Immunoassay Cellular System – Set 1** in combination with the anti ER antibodies described in Table 1.

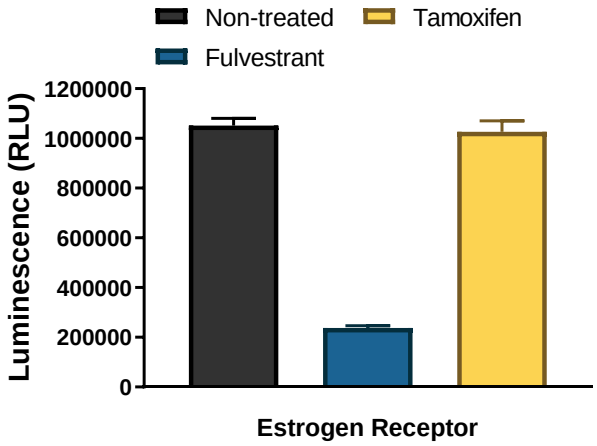

Figure 2. Detection of total ER protein using the Lumit™ Immunoassay Cellular System – Set 1. 50,000 seeded MCF-7 cells were starved overnight. The cells were then untreated or treated with Fulvestrant or Tamoxifen (50nM) for 4 hours. Total Estrogen receptor levels were measured following Promega Technical Manual TM613 and using the primary antibody conditions described in Table 1.

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Activation of Estrogen Receptor Degradation with Fulvestrant

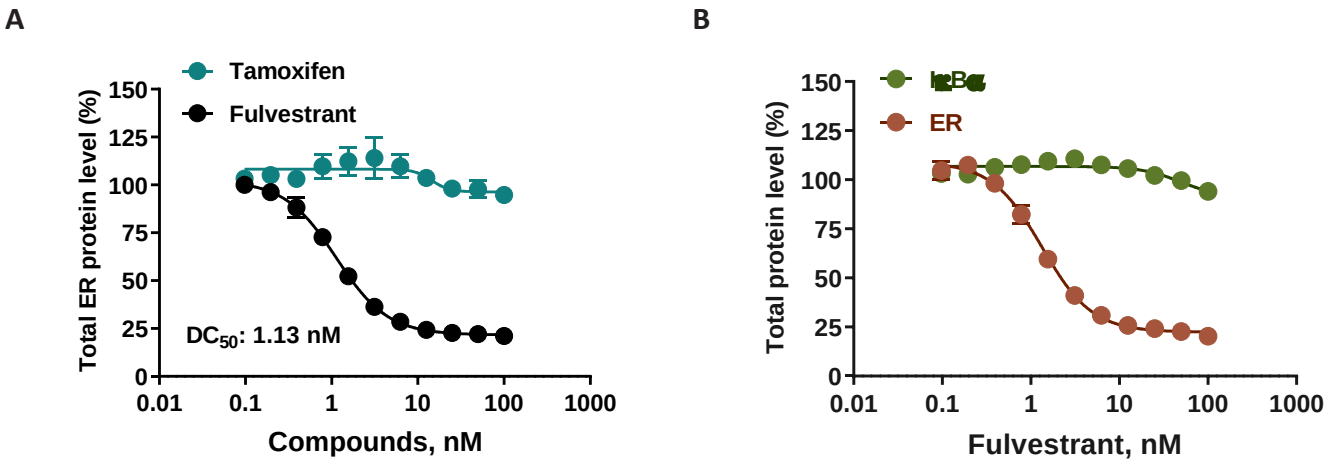

Figure 3. Targeted degradation of Estrogen Receptor with small molecule degrader. After starvation overnight, 50,000 seeded MCF-7 cells were treated with various concentrations of Fulvestrant or Tamoxifen for 4 hours before ER (A) or ER and IκBα (B) were measured by **Lumit™ Immunoassay Cellular System – Set 1** to determine the potency of the small molecules (DC_{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)
ER (Rabbit)	Total	Thermo Fisher Scientific	MA5-14501	Supplied at stock concentration
ER (Mouse)	Total	Thermo Fisher Scientific	MA5-13191	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 1	100 assays	W1201
	1,000 assays	W1202
	10,000 assays	W1203