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).


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 (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.

Figure 1. Illustration of Lumit™ Cellular Immunoassay. When the primary antibody pair includes a phosho-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.
Lumit™ Immunoassay Cellular System Application Note
Cellular Pathway Analysis Series

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 (DC50).

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>
<thead>
<tr>
<th>Antibody*</th>
<th>Target</th>
<th>Supplier</th>
<th>Cat. #</th>
<th>Working stock (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (Rabbit)</td>
<td>Total</td>
<td>Thermo Fisher Scientific</td>
<td>MA5-14501</td>
<td>Supplied at stock concentration</td>
</tr>
<tr>
<td>ER (Mouse)</td>
<td>Total</td>
<td>Thermo Fisher Scientific</td>
<td>MA5-13191</td>
<td>50</td>
</tr>
</tbody>
</table>

*Antibodies from other suppliers may work as well. They may need optimization following Promega Technical Manual TM613.

Ordering Information:

<table>
<thead>
<tr>
<th>Products</th>
<th>Size</th>
<th>Promega Cat. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumit™ Immunoassay Cellular System – Set 1</td>
<td>100 assays</td>
<td>W1201</td>
</tr>
<tr>
<td></td>
<td>1,000 assays</td>
<td>W1202</td>
</tr>
<tr>
<td></td>
<td>10,000 assays</td>
<td>W1203</td>
</tr>
</tbody>
</table>