**Luciferase Cell Lysis Buffer**

25 ml Lot: 0041205 Exp: 5/15

5X buffer Store at 4°C

Description: Luciferase Cell Lysis Buffer (LCLB) is a proprietary formulation developed to produce mammalian cell lysates for reporter assays. This lysis buffer is compatible with reagents for assaying the activity of Gaussia as well as other luciferases (e.g. Renilla & Firefly) and ß-galactosidase (Figure 1A-D).

**Protocol:**
1. Dilute LCLB (5X) with dH2O to 1X concentration.
2. Aspirate the growth media from wells.
3. Wash the cells once with PBS (pH 7.4) and aspirate.
4. Add the appropriate volume of 1X LCLB to each well (See Table 1).
5. Incubate at room temp for 15–20 min on an orbital shaker (making sure the surface in a well is completely covered with the buffer).
6. Use 5–20 µL of cell lysate for assaying.

**Table 1: Recommended LCLB Volumes**

<table>
<thead>
<tr>
<th>Culture Vessel</th>
<th>Surface (cm²)</th>
<th>Volume of 1X LCLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 well</td>
<td>0.32</td>
<td>25 µL</td>
</tr>
<tr>
<td>24 well</td>
<td>0.95</td>
<td>75 µL</td>
</tr>
<tr>
<td>12 well</td>
<td>1.9</td>
<td>150 µL</td>
</tr>
<tr>
<td>35 mm dish</td>
<td>3.8</td>
<td>250 µL</td>
</tr>
<tr>
<td>6 well</td>
<td>9.5</td>
<td>800 µL</td>
</tr>
<tr>
<td>60 mm dish</td>
<td>21</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>100 mm dish</td>
<td>55</td>
<td>2.5 ml</td>
</tr>
</tbody>
</table>

**Notes On Use:**
1. Depending on the cell type, some cells may need larger volume and longer incubation time for sufficient lysis. One can determine whether or not the lysis is sufficient by assaying the activity of intracellular Gaussia luciferase using the pCMV-GLuc Control Plasmid (NEB #N8081). Although the Gaussia luciferase produced from pCMV-GLuc is secreted into the culture medium, at a given time point, 5-10% of the total activity should reside in the lysate (Figure 1A).

2. Normally, there is no need to remove the cell debris by centrifugation before assaying Gaussia luciferase activity. If additional assays are to be performed (e.g. protein concentration, SDS-PAGE, etc.), it is best to centrifuge the cell lysate to remove cell debris.

**Companion Products:**
- pCMV-GLuc Control Plasmid #N8081S 20 µg
- pGLuc Basic Vector #N8082S 20 µg
- Gaussia Luciferase Assay Kit #E3300S 100 assays #M3300L 1,000 assays
- TransPass™ D2 Transfection Reagent #M2554S 0.6 ml #M2554L 3.0 ml
- TransPass™ COS/293 Transfection Reagent #M2557S 1.2 ml
- TransPass™ HUVEC Transfection Reagent #M2558S 1.8 ml
- TransPass™ R1 Transfection Reagent #M2551S 0.4 ml
- TransPass™ HeLa Transfection Reagent #M2556S 0.8 ml

**Figure 1. LCLB compatibility with commonly used reporter assay systems.** NIH3T3 cells were transfected either with (A) pCMV-GLuc Control Plasmid (NEB #N8081S), (B) a lacZ-expressing vector, (C) a Renilla-expressing vector or (D) a Firefly-expressing vector, using TransPass D2 Transfection Reagent (NEB #M2554S). The supernatants from CMV-GLuc-transfected cells were saved for the assay at 24 h after transfection. All transfected cells were then washed once with PBS (pH 7.4) and lysed with either Luciferase Cell Lysis Buffer or commercially available lysis buffers recommended for each reporter system, respectively. Cell lysates (20 µL out of 100 µL total lysate per sample) were analyzed for GLuc or Renilla luciferase activity using the GLuc Assay Kit (NEB #E3300S/L), ß-galactosidase activity using Galacto-Light System (ABI), and Firefly luciferase activity using Luciferase Assay System (Promega). Gaussia luciferase activity was also assayed from the supernatants (20 µL out of 500 µL growth medium of CMV-GLuc-transfected cells per sample).