Reagent Preparation

1. **1X PLB:** Add 1 volume of 5X Passive Lysis Buffer (PLB) to 4 volumes of distilled water. Mix well. Store at 4°C (≤ 1 month).

2. **LAR II:** Resuspend the lyophilized Luciferase Assay Substrate in Luciferase Assay Buffer II (10ml for Cat.# E1910, E1960; 105ml for Cat.# E1980). Store at −20°C (≤ 1 month) or −70°C (≤ 1 year).

3. **Stop & Glo® Reagent:**
   a. Add 2.1ml of 50X Stop & Glo® Substrate to 105ml of Stop & Glo® Buffer in the amber Stop & Glo® Reagent bottle provided. Vortex 10 seconds. Store at −20°C for 15 days.
   b. For a smaller amount of **1X Stop & Glo® Reagent:** To the required amount of Stop & Glo® Buffer, add 50X Stop & Glo® Substrate to a final 1X concentration. (For example, add 0.2ml of 50X Stop & Glo® Substrate to 10ml of Stop & Glo® Buffer to make a 1X solution of Stop & Glo® Reagent.)

Cell Lysis

1. Remove growth media from cultured cells.
2. Rinse cultured cells in 1X PBS. Remove all rinse solution.
3. Dispense the recommended volume (below) of 1X PLB into each culture vessel.

<table>
<thead>
<tr>
<th>Volumes of 1X PLB to Use in Step 3.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Passive Lysis</strong></td>
</tr>
<tr>
<td>Plate Size</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>6-well</td>
</tr>
<tr>
<td>12-well</td>
</tr>
<tr>
<td>24-well</td>
</tr>
<tr>
<td>48-well</td>
</tr>
<tr>
<td>96-well</td>
</tr>
</tbody>
</table>

4. **Passive Lysis:** Gently rock/shake the culture vessel for 15 minutes at room temperature. Transfer lysate to a tube or vial.*

*For automated applications, the DLR™ Assay is performed directly in the multiwell plate.

Additional protocol information is available in Technical Manual #TM040 or #TM046, available online at: www.promega.com
Dual-Luciferase® and Dual-Luciferase® 1000 Assay Protocols

**Assay with 96-Well Plate**

Before you begin:

Set injectors 1 and 2 to dispense 100µl of LAR II and Stop & Glo® Reagent, respectively.

For measurements, use a 1- to 2-second delay and a 5- to 10-second read time.

(inside luminometer)

- Plate with ≤20µl of PLB Lysate/well.
- Dispense 100µl of LAR II.
- Measure firefly luciferase activity.
- Dispense 100µl of Stop & Glo® Reagent.
- Measure Renilla luciferase activity.
- Repeat cycle for remaining wells in plate.

**Assay with Manual or Single-Injector Luminometer**

- Predispense 100µl of LAR II into luminometer tube.
- Program luminometer.
- Transfer 20µl of PLB Lysate. Mix.
- Measure firefly luciferase activity.
- Dispense 100µl of Stop & Glo® Reagent.
- Measure Renilla luciferase activity.

Additional protocol information is available in Technical Manual #TM040 or #TM046, available online at: www.promega.com

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