Reagent Preparation

1. Allow all reagents to reach room temperature (20 – 22°C).

2. **Passive Lysis Solution**: PLS is a ready to use reagent.

3. **firelite plus reagent**:
   - Reconstitute one vial firelite plus Lyophilized Substrate with 53 mL of firelite plus Reconstitution Buffer.
   - Mix the contents of the vial gently by inversion and leave for 5 minutes.
     - Unused reagent can be stored at –20°C (≤ 2 months) or –80°C (≤ 2 year).

4. **renlite plus reagent**:
   - Add 2.12 mL renlite plus Substrate (50X) to 106 mL renlite plus Buffer.
   - When a smaller quantity of renlite plus reagent is desired, add an appropriate volume of renlite plus Substrate (50X) to the required amount of renlite plus Buffer to achieve the correct concentration of 1X renlite plus Substrate. For example, add 300 µL renlite plus Substrate (50X) to 15 mL renlite plus Buffer.
   - Mix the contents of the bottle gently by inversion and leave for 5 minutes.
     - Unused reagent can be stored at –20°C (≤ 3 months) or –80°C (≤ 2 year).

Cell Lysate Preparation

1. Remove cell growth medium from the cell layer.

2. Wash the cells with a sufficient amount of PBS at room temperature. Swirl briefly to remove loose cells and residual growth medium. Remove the wash solution as much as possible.

3. Add to the cell layer the recommended volume of PLS according to the table below.

<table>
<thead>
<tr>
<th>Plate type</th>
<th>PLS/well</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 well</td>
<td>500 µL</td>
</tr>
<tr>
<td>12 well</td>
<td>250 µL</td>
</tr>
<tr>
<td>24 well</td>
<td>100 µL</td>
</tr>
<tr>
<td>48 well</td>
<td>65 µL</td>
</tr>
<tr>
<td>96 well</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

4. Place the plate on an orbital shaker or on a rocking platform so that the PLS covers the cell layer evenly for optimal lysis. Shake the plate for 15 minutes at room temperature.

5. The cell lysate can now be used in the twinlite assay. If the lysate is not needed the same day, store at –20°C (≤ 2 months) or –80°C (≤ 1 year). When the cells are cultured in an opaque 96-well plate, then the assay can be performed directly in the same plate without lysate transfer.
**Measuring firefly and renilla luciferase luminescence; twinlite assay**

1. Set the luminometer injectors 1 and 2 to dispense 100 μL **firelite plus** and **renlite plus** reagent respectively.

2. Set a count delay of 2 seconds between the reagent injection and measuring luminescence. Set the luminescence read time between 5 to 10 seconds.

3. Fill and rinse the designated injectors of the luminometer with the prepared reagents.

4. Load the microplate containing the samples (20 μL/well) in the luminometer, dark adapt for a few minutes to decrease plate phosphorescence (to lower plate background levels) and start the measurement.

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**Assay using a 96-well plate (opaque)**

96-well plate with 20 μL PLS lysate/well.

Inject 100 μL of **firelite plus**
*Measure firefly luciferase luminescence for 5 – 10 seconds after a 2 seconds count delay.*

Inject 100 μL of **renlite plus**
*Measure renilla luciferase luminescence for 5 – 10 seconds after a 2 seconds count delay.*

Repeat this cycle for the other samples in the plate.

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This product and/or its use is covered by the following patents and corresponding patent applications worldwide, owned by PerkinElmer Health Sciences B.V.: US Patent No. 8,512,968; EP Patent No. EP2222870B1; China Patent No. CN101889095B; and Australia Patent No. AU2008319571B2.