Dual-Luciferase® Reporter Assay and Dual-Luciferase® Reporter 1000 Assay Systems

Quick Protocol

Instructions for Use of Products E1910, E1960 and E1980.

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).

   Note: The lysis buffer color may vary due to a naturally derived raw material in the formulation. This color variation does not affect product performance.

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).

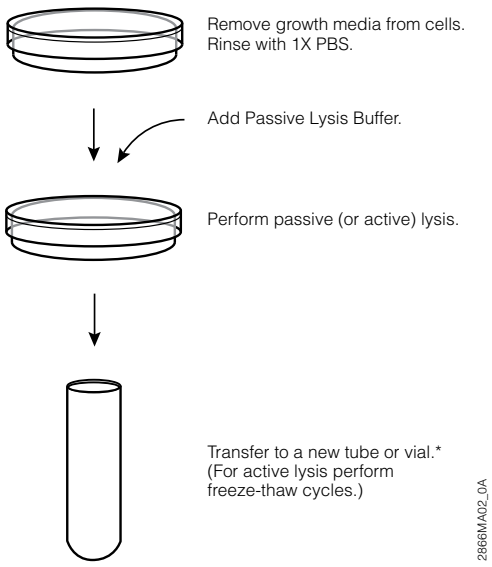
3. **Stop & Glo® Reagent:**
   a. Add 2.1ml of **50X Stop & Glo® Substrate** to 105ml of **Stop & Glo® Buffer** in the amber **Stop & Glo® Reagent** bottle provided. Vortex 10 seconds. Store at –20°C for 15 days.
   b. For a smaller amount of **1X Stop & Glo® Reagent:** To the required amount of **Stop & 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 (see table) of **1X PLB** into each culture vessel.

Volumes of 1X PLB to Use in Step 3.

Passive Lysis		Active Lysis	
Plate Size	**1X PLB**	**Dish/Plate Size**	**1X PLB**
6-well	500µl	100 × 200mm	1ml
12-well	250µl	60 × 15mm	400µl
24-well	100µl	35 × 12mm	200µl
48-well	65µl	6-well	250µl
96-well	20µl	12-well	100µl



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

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Dual-Luciferase® and Dual-Luciferase® 1000 Assay Protocols

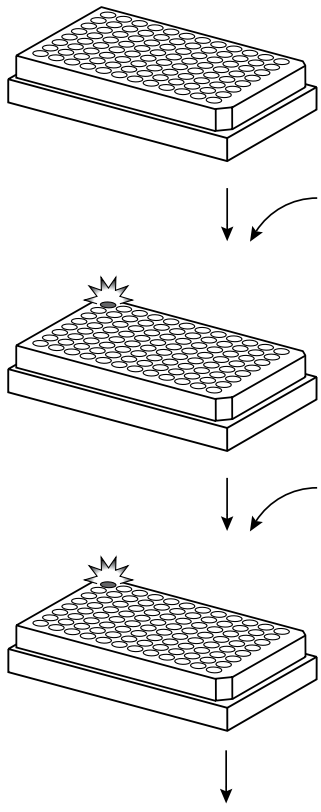
Assay with 96-Well Plate

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)



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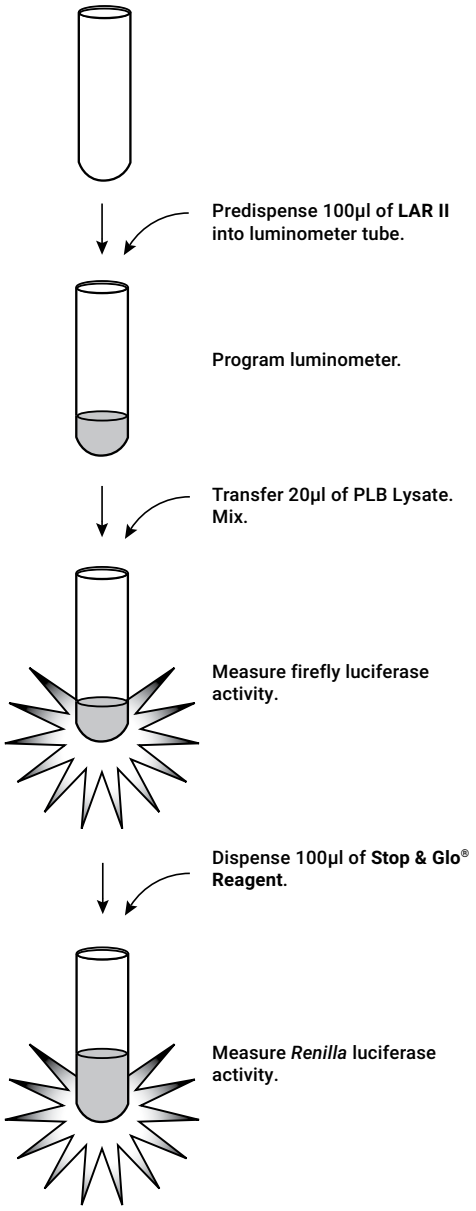
Assay with Manual or Single-Injector Luminometer



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