

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

INSTRUCTIONS FOR USE OF PRODUCTS E1910, E1960 AND E1980.

Quick
PROTOCOL

Reagent Preparation

- 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).
- 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).
- Stop & Glo[®] Reagent:**
 - 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.
 - 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

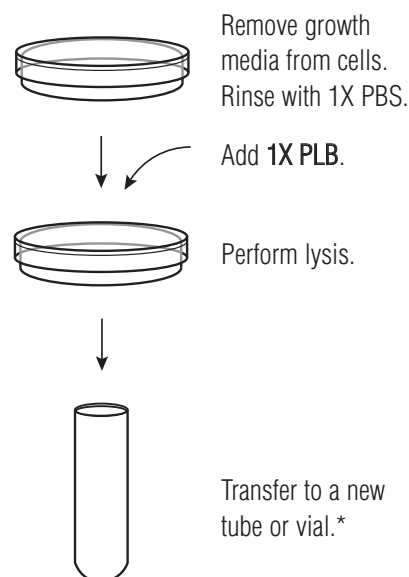
- Remove growth media from cultured cells.
- Rinse cultured cells in 1X PBS. Remove all rinse solution.
- Dispense the recommended volume (below) of **1X PLB** into each culture vessel.

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

- 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.

Cell Lysis



2866MA02_0A

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

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

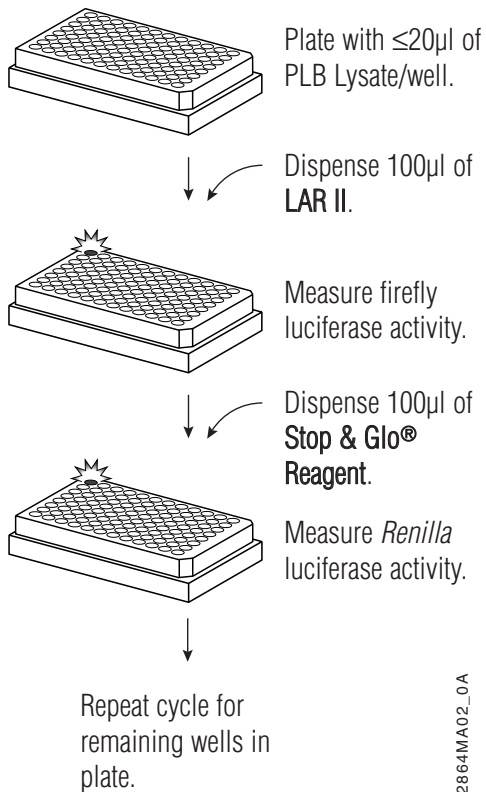
Assay with 96-Well Plate

Before you begin:

Set injectors 1 and 2 to dispense 100 μ l of **LAR II** and **Stop & Glo[®] Reagent**, respectively.

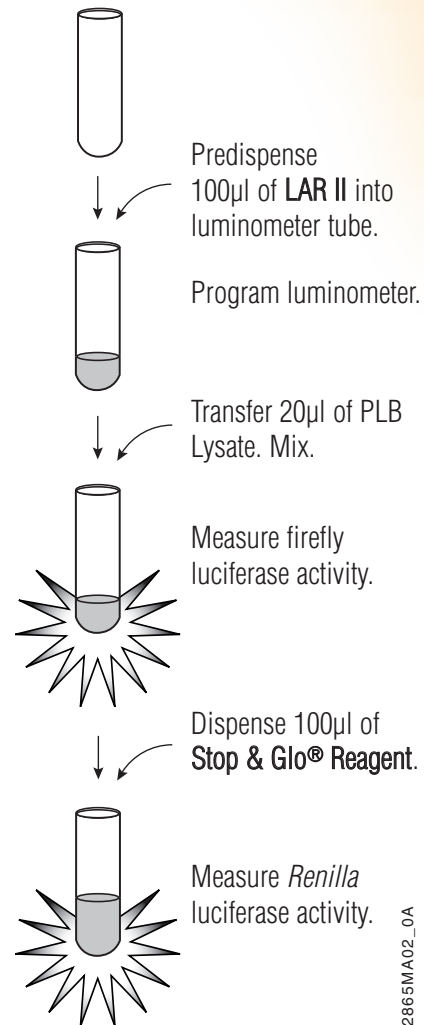
For measurements, use a 1- to 2-second delay and a 5- to 10-second read time.

(inside luminometer)



2864MA02_0A

Assay with Manual or Single-Injector Luminometer



2865MA02_0A

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