

# Dual-Luciferase<sup>®</sup> Reporter Assay and Dual-Luciferase<sup>®</sup> Reporter 1000 Assay Systems

Quick Protocol

Instructions for Use of Products E1910, E1960 and E1980.

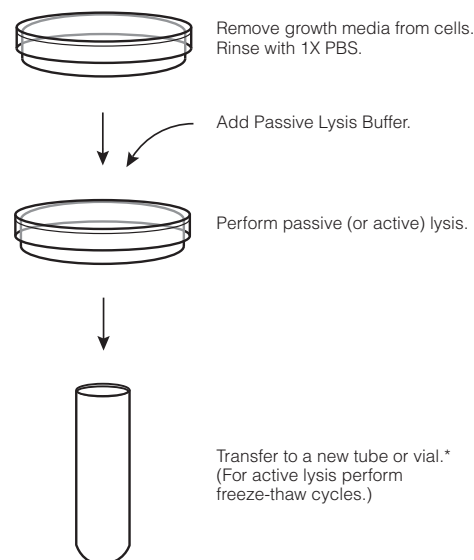
## Reagent Preparation

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

## Cell Lysis

- Remove growth media from cultured cells.
- Rinse cultured cells in 1X PBS. Remove all rinse solution.
- 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



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- 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.

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## Dual-Luciferase<sup>®</sup> and Dual-Luciferase<sup>®</sup> 1000 Assay Protocols

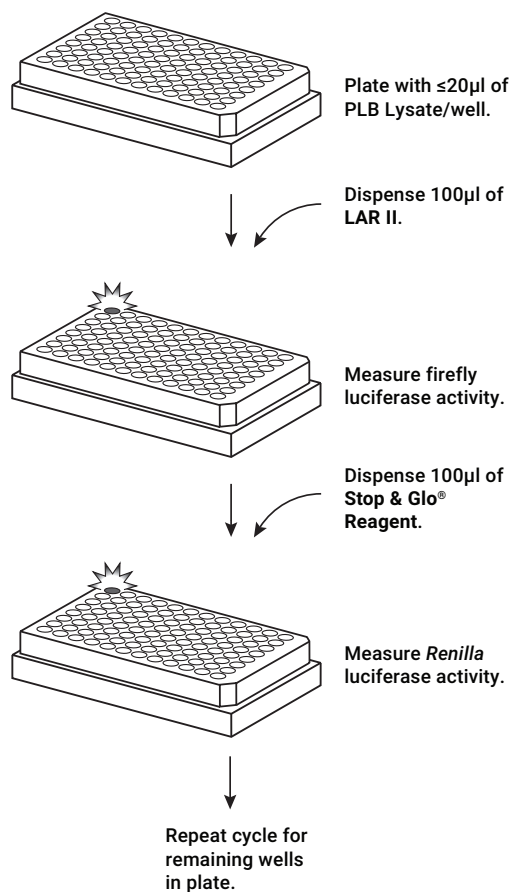
### Assay with 96-Well Plate

#### Before you begin:

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

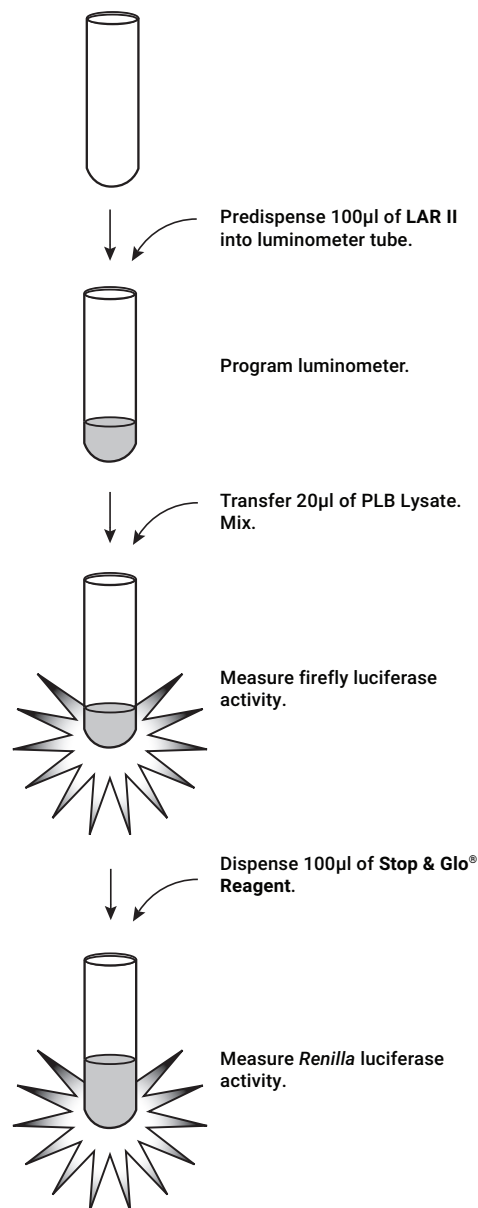
For measurements, use a 1- to 2-second delay and a 5- to 10-second read time.

(Inside Luminometer)



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### Assay with Manual or Single-Injector Luminometer



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Additional protocol information in Technical Manual #TM040 or #TM046, available online at: [www.promega.com](http://www.promega.com)