

Dual-Glo[®] Luciferase Assay System

Instructions for Use of Products E2920, E2940 and E2980.

Quick Protocol

Dual-Glo[®] Luciferase Assay System is designed for use in mammalian cell culture medium particularly with the following types of medium containing 1–10% serum: RPMI 1640, DMEM, MEMα and F12.

Preparation of Reagents

1. Transfer the contents of one bottle of Dual-Glo[®] Luciferase Buffer to one bottle of Dual-Glo[®] Luciferase Substrate to make Dual-Glo[®] Luciferase Reagent.
2. Calculate the amount of Dual-Glo[®] Stop & Glo[®] Reagent needed for the experiment. Using a new container, dilute the Dual-Glo[®] Stop & Glo[®] Substrate 1:100 into Dual-Glo[®] Stop & Glo[®] Buffer to make the needed volume of Dual-Glo[®] Stop & Glo[®] Reagent.

Notes:

- a. Assay reagents are stable at room temperature for several hours. The Dual-Glo[®] Luciferase Assay Reagent may be frozen to prevent loss of activity but should **not** be thawed at temperatures above 25°C. Thaw in a room temperature water bath. Mix well after thawing.
- b. Dual-Glo[®] Luciferase Reagent Stability: Liquid reagent will lose approximately 10% of firefly relative light units (RLU) after 8 hours at room temperature and after 48 hours at 4°C. Frozen reagent will lose approximately 10% of firefly RLU after 1 week at –20°C and after 6 months at –70°C. Do not store reagent at –20°C for longer than one week.
- c. Stability of Dual-Glo[®] Stop & Glo[®] Reagent after reconstitution: 8.1% loss of activity after 8 hours at room temperature; 8.5% loss after 24 hours at 4°C. We recommend that Dual-Glo[®] Stop & Glo[®] Reagent always be prepared immediately before use.
- d. The temperature for optimal activity of both firefly and *Renilla* luciferase is room temperature (20–25°C), thus reagents should be equilibrated to room temperature before use. Equilibrate cells in medium to room temperature before performing luciferase measurements.

(continued, next page)

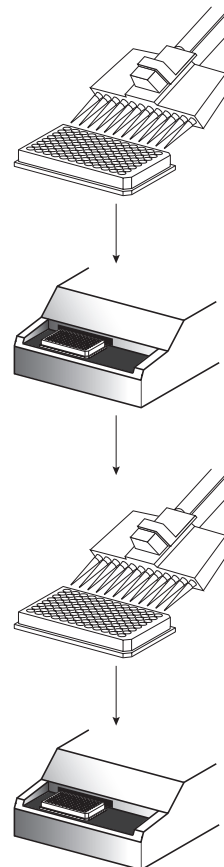
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1. Remove 96- or 384-well plates containing mammalian cells from the incubator. The plates used must be compatible with the luminometer being used. For best results, equilibrate cultured cells to room temperature before performing Step 2.
2. To each plate well add a volume of Dual-Glo[®] Reagent equal to the volume of culture medium in the well and mix. (For 96-well plates, add 75µl of Reagent to cells grown in 75µl of medium. For 384-well plates, add 20µl of Reagent to cells grown in 20µl of medium.)
3. Wait at least 10 minutes to allow for cell lysis to occur, then measure the **firefly luminescence** in a luminometer.
4. Add a volume of Dual-Glo[®] Stop & Glo[®] Reagent equal to the original culture medium volume to each well and mix. As noted in Step 2, this volume is typically 75µl for 96-well plates and 20µl for 384-well plates.
Note: Dual-Glo[®] Stop & Glo[®] Reagent should be added to plate wells within 4 hours of addition of Dual-Glo[®] Luciferase Assay Reagent.
5. Wait at least 10 minutes, then measure **Renilla luminescence**. The *Renilla* luminescence should be read in the same plate order as the firefly luminescence.
6. Calculate the ratio of luminescence from the experimental reporter to luminescence from the control reporter. Normalize this ratio to the ratio of a control well or series of control wells that have been treated consistently on all plates. This normalization provides optimal and consistent results from the Dual-Glo[®] Luciferase Assay System.
7. Calculate Relative Response Ratios from the normalized ratios. See Section 6.C of the *Dual-Glo[®] Luciferase Assay System Technical Manual*, #TM058, for more information and sample calculations.



Add Dual-Glo[®] Luciferase Assay Reagent to the plate.

Incubate at 20–25°C for 10 minutes–2 hours.

Measure firefly luminescence.

Add Dual-Glo[®] Stop & Glo[®] Reagent to the plate.

Incubate at 20–25°C for 10 minutes–2 hours.

Measure *Renilla* luminescence.

Calculate ratio of firefly:*Renilla* luminescence for each well. Normalize the sample well ratio to the ratio from a control (or series of control) well(s).

08/11/14

For additional protocol information consult Technical Manual #TM058, available online at: www.promega.com