Certificate of Analysis

Glo Lysis Buffer:

Part# 9PIE266

Revised 10/16

Part No.	Size
E266A	100ml

Description: Glo Lysis Buffer (GLB), 1X is a proprietary formulation developed to promote rapid lysis (within 5 minutes) of cultured mammalian cells without scraping or performing freeze-thaw cycles. It is fully compatible with Bright-Glo™, Steady-Glo®, ONE-Glo™ and *Renilla*-Glo™ Luciferase Assay Reagents for analysis of luciferase expression. The half-life of these reagents remains the same with or without the use of GLB; it is >5 hours for Steady-Glo® Reagent and >24 minutes for Bright-Glo™ Reagent.

Firefly luciferase is stable for at least 48 hours at room temperature when diluted to 2.2×10^{-10}M in 1X Glo Lysis Buffer containing 1mg/ml BSA (Figure 1).

GLBuffer expands the potential uses of Bright-Glo™, Steady-Glo®, ONE-Glo™ and *Renilla*-Glo™ Luciferase Assay Reagents. These products are designed for homogeneous assays; however, GLB enables them to be used in nonhomogeneous formats.

Storage Conditions: See the product information label for storage recommendations. For long-term storage, Glo Lysis Buffer can be frozen at –20°C or –70°C. See the product information label for the expiration date.

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Quality Control Assays

When Glo Lysis Buffer is used as instructed in the *Steady-Glo® Luciferase Assay System Technical Manual*, #TM051, the half-life of the luciferase signal is ≥5 hours.

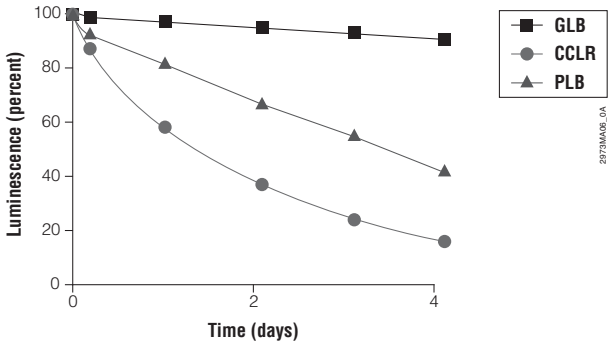

Figure 1. Stability of firefly luciferase in Glo Lysis Buffer. Purified firefly luciferase (2.2×10^{-10}M with 1mg/ml BSA) was added to Glo Lysis Buffer (GLB), Cell Culture Lysis Reagent (CCLR) or Passive Lysis Buffer (PLB). Luciferase was incubated at 22°C for up to 100 hours with samples taken at 24-hour intervals and stored at –70°C. After 100 hours, all samples were thawed and initial luminescence measured. Luminescent reactions were initiated by adding 100µl of the respective lysis reagent to Bright-Glo™ Assay Reagent. Stability was determined as the amount of time necessary for initial luminescence to decrease to 90% of the luminescence at time zero.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. Glo Lysis Buffer Protocol

Glo Lysis Buffer (GLB) should be used in a 1:1 ratio with reconstituted Steady-Glo® or Bright-Glo™ Assay Reagents following the protocol below.

A. GLB Protocol

1. Equilibrate the Glo Lysis Buffer to room temperature before use.
2. Equilibrate the cells to room temperature. Aspirate the medium from the cells. Gently rinse with 1X PBS (cell rinse is optional).
3. Add a sufficient volume of Glo Lysis Buffer to the sample well or plate to cover cells (see Table 1). When using lysate with other applications, account for the volume needed for that application.
4. Rock the plate slowly several times to ensure complete coverage of the cells with Glo Lysis Buffer.
5. Incubate for 5 minutes at room temperature to allow lysis to occur.
6. Transfer the lysate to luminometer tubes, plate wells or vials and add Steady-Glo® or Bright-Glo™ Assay Reagent (Glo Lysis Buffer can be used in a 1:1 ratio with reconstituted Steady-Glo® or Bright-Glo™ Assay Reagent). Wait 5 minutes, then measure luminescence with a luminometer following the manufacturer's instructions.

   Note: Lysate prepared with Glo Lysis Buffer can be stored at –20°C or –70°C and is stable over several freeze-thaw cycles.

B. Preparing Firefly Luciferase Positive Controls

To prepare a positive control, dilute QuantiLum® Recombinant Luciferase enzyme (Cat.# E1701) in 1X Glo Lysis Buffer, containing 1mg/ml BSA. Equilibrate the luciferase to room temperature for 20 minutes before performing an assay.

Alternatively, the QuantiLum® Recombinant Luciferase can be serially diluted in 1X Glo Lysis Buffer containing 1mg/ml BSA, then diluted 1:10 in cell culture medium.

To perform a firefly luciferase activity assay, add to 100µl of QuantiLum® Recombinant Luciferase, diluted as indicated above, to 100µl of Steady-Glo® or Bright-Glo™ Assay Reagent. For specific QuantiLum® Luciferase dilution instructions see either the *Steady-Glo® Luciferase Assay System Technical Manual*, #TM051, or the *Bright-Glo™ Luciferase Assay System Technical Manual*, #TM052.

Note: Enzyme dilutions can be prepared and stored for up to 3 months at –80°C. Prepare by diluting in Glo Lysis Buffer to 10^{-3} to 10^{-6} and store in 500µl aliquots.

Table 1. Amount of Glo Lysis Buffer for Use with Various Plate Sizes.

Plate	Glo Lysis Buffer
100mm	3ml
60mm	1.1ml
35mm	500µl
6 wells	500µl
12 wells	200µl
24 wells	100µl
96 wells	100µl

2. Related Products

Luciferase Assay Systems

Product	Size	Cat.#
Steady-Glo® Luciferase Assay System	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
Bright-Glo™ Luciferase Assay System	10ml	E2610
	100ml	E2620
	10 × 100ml	E2650
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-Pack	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System	1,000 assays	E1980
Luciferase Assay System	100 assays	E1500
Luciferase Assay System with Reporter Lysis Buffer	100 assays	E4030
Luciferase Assay Reagent, 10-Pack	1,000 assays	E1501
Luciferase Assay System Freezer Pack	1,000 assays	E4530
Luciferase 1000 Assay System	1,000 assays	E4550
Luciferase Assay Reagent	1,000 assays	E1483

Luciferase Reporter Vectors

Product	Size	Cat.#
pGL3-Control DNA	20µg	E1741
pGL3-Basic DNA	20µg	E1751
pGL3-Promoter DNA	20µg	E1761
pGL3-Enhancer DNA	20µg	E1771

Miscellaneous Luciferase Products

Product	Size	Cat.#
QuantiLum® Recombinant Luciferase	1mg	E1701
	5mg	E1702

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