Revised: January 3, 2013

Product Information

5X Firefly Lysis Buffer

Catalog Number: 99923

Unit Size: 15 mL

Storage and Handling

Store at –20°C or below. The buffer is stable for at least 12 months from date of receipt when stored as recommended.

Product Description

5X Firefly Lysis Buffer is a concentrated cell lysis buffer that is designed for use with Biotium's Firefly Luciferase Assay Kit (catalog no. 30003) and Firefly Luciferase Assay Kit (Lyophilized) (catalog no. 30075). These kits are supplied with sufficient 5X Firefly Lysis Buffer to perform the stated number of assays with cells grown in 96 – 24 well plates. 5X Firefly Lysis Buffer is sold separately for use with the kits when more lysis buffer (e.g. >100 uL/well) is required.

Note: 5X Firefly Lysis Buffer is not compatible with Renilla Luciferase. If you require additional lysis buffer for use with Biotium's Renilla Luciferase Assay Kit (catalog no. 30004) or Firefly and Renilla Luciferase Assay Kit (catalog no. 30005), order 5X Passive Lysis Buffer (catalog no. 99912).

Protocol

Preparation of Cell Lysates

Preparation of Firefly Luciferase Lysis Buffer

1. Prepare 1X lysis buffer by adding 1 volume of 5X Firefly Luciferase Lysis Buffer to 4 volumes of dH_2O and mixing well. 1X lysis buffer may be stored at 4°C for up to one month.

Lysis of Cells Cultured in Multiwell Plates

1. Remove growth medium from cultured cells and gently add a sufficient volume of phosphate buffered saline (PBS) to wash the surface of the culture vessel. Add 1X lysis buffer to each well using the volume recommended below for each type of culture plate:

Wells/plate	Lysis buffer/well
6 well	500 uL
12 well	250 uL
24 well	100 uL
48 well	65 uL
96 well	20 uL

2. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X lysis buffer. Rock the culture plates at room temperature for 15 minutes.

   Note: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of lysis buffer and/or an extended treatment period to ensure complete lysis. Lifting cells from the plate will facilitate the process of cell lysis. Biotium offers mini cell scrapers (cat. no. 22003) for harvesting lysates from 96-, 24-, and 48-well plates.

3. Transfer the lysate to a tube or vial. Optional: the lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube. Place at 4°C for until ready to assay. Store lysates at -20°C or -70°C if assay will not be performed on the same day.

4. To assay lysates with Biotium's Firefly Luciferase Assay Kit (catalog no. 30003) or Firefly Luciferase Assay Kit (Lyophilized) (catalog no. 30075), follow the assay protocols provided with the kit.

Related Products

Catalog number	Product
22003	Mini Cell Scrapers, pack of 200
30003	Firefly Luciferase Assay Kit
30075	Firefly Luciferase Assay Kit (Lyophilized)
30004	Renilla Luciferase Assay Kit
30005	Firefly & Renilla Dual Luciferase Assay Kit
99912	5X Passive Lysis Buffer
30028	Steady-Luc Firefly HTS Assay Kit
30020	ATP-Glo Bioluminometric Cell Viability Assay

Please visit our website at www.biotium.com for information on our life science research products, including apoptosis and cell viability assays, fluorescent CF™dye antibody conjugates and reactive dyes, environmentally friendly EvaGreen® qPCR master mixes, and many other products for life science research.

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