

# Product Information

## 5X Passive Lysis Buffer

**Catalog Number:** 99912

**Unit Size:** 30 mL

### Storage and Handling

Store at  $-20^{\circ}\text{C}$  or below. The buffer is stable for at least 12 months from date of receipt when stored as recommended.

### Product Description

5X Passive Lysis Buffer is a concentrated cell lysis buffer that is designed for use with Biotium's Renilla Luciferase Assay Kit (catalog no. 30004) and Firefly and Renilla Luciferase Assay Kit (catalog no. 30005). These kits are supplied with sufficient 5X Passive Lysis Buffer to perform the stated number of assays with cells grown in 96 – 24 well plates. 5X Passive Lysis Buffer is sold separately for use with the kits when more lysis buffer (e.g.  $>100\ \mu\text{L}/\text{well}$ ) is required.

### Protocol

#### Preparation of cell lysates

Preparation of 1X Passive Lysis Buffer

1. Prepare 1X Passive Lysis Buffer by adding 1 volume of 5X passive lysis buffer to 4 volumes of  $\text{dH}_2\text{O}$  and mixing well. 1X passive lysis buffer may be stored at  $4^{\circ}\text{C}$  for up to one month.

Lysis of Cells Cultured in Multiwell Plates

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

Wells/plate	Lysis buffer/well
6 well	500 $\mu\text{L}$
12 well	250 $\mu\text{L}$
24 well	100 $\mu\text{L}$
48 well	65 $\mu\text{L}$
96 well	20 $\mu\text{L}$

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 passive 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 passive lysis buffer, extended treatment period, and/or scraping cells off the culture plates to ensure complete lysis. Biotium offers mini cell scrapers (catalog 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  $4^{\circ}\text{C}$  for until ready to assay. Store lysates at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$  if assay will not be performed on the same day.
4. To assay lysates with Biotium's Renilla Luciferase Assay (catalog no. 30004) or Firefly and Renilla Luciferase Assay Kit (catalog no. 30005), follow the assay protocols provided with the kit.

### Related Products

Catalog number	Product
22003	Mini Cell Scrapers, pack of 200
30004	Renilla Luciferase Assay Kit
30005	Firefly & Renilla Dual Luciferase Assay Kit
30003	Firefly Luciferase Assay Kit
30075	Firefly Luciferase Assay Kit (Lyophilized)
99923	5X Firefly Luciferase Lysis Buffer
30028	Steady-Luc Firefly HTS Assay Kit
30020	ATP-Glo Bioluminometric Cell Viability Assay

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