# *BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO*

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## Luciferase (LUC) Assay Kit (Cat #: E-126)

**COMPONENTS**: Luciferase Assay Solution- 2x10 ml (200 assays), store at -80ºC **(shield solution from light during assay)**
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

**PRODUCT DESCRIPTION:** Luciferase catalyzes in the presence of ATP the oxidation of luciferin with concomitant emission of yellow-green light, which can be conveniently measured by a luminometer. Light emission peaks in several seconds at 560 nm when the reaction is conducted at pH 7.8 (Anal. Biochem. 80:496,1977). Rapid appearance and decay of the light flash require consistent timing of the light measurement to obtain reliable data. This provides the basis for an assay system much more sensitive than the β-galactosidase (GAL) assay and chloramphenicol acetyltransferase (CAT) assay. The Cell Lysis Solution is compatible with both GAL and CAT assays, thus allowing for dual assays to be performed from the same cell extract. Kit components are stable for many years if stored and handled properly.

**PROTOCOL:**

### Luciferase Assay Solution

Thaw Luciferase Assay Solution quickly. *Do not over thaw.* Keep solution on ice and shielded from light. Freeze solution immediately after use.

### Dilution of 10x Cell Lysis Solution

Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold $dH_2O$. Place 1x Cell Lysis Solution on ice.

### Preparation of cell lysates:

1. The protocol is for adherent cells plated on 35-mm dishes and can be scaled up or down proportionally for culture vessels of different size. Aspirate off culture medium, rinse cells with phosphate-buffered saline (PBS) twice and aspirate off solution completely.

2. To each 35-mm dish, add 100 µl ice-cold 1x Cell Lysis Solution. Scrape cells off with a cell scraper. Transfer cell lysate to a microtube kept on ice. Gently pipette solution up and down to break up cell aggregates if necessary. Clarify lysate in a refrigerated microfuge at ~14,000 rpm for 5 min.

3. Pipet 10 − 20 µl of each supernatant to a plain (uncoated) 96-well plate. Add 100 µl Luciferase Assay Solution to each well and measure relative light unit (RLU) using a luminometer.

4. Background RLU is measured by using 10 − 20 µl 1x Cell Lysis Solution. Subtract background RLU from sample RLU.

5. Sample RLU may be normalized by protein centration or another reporter enzyme.