BIOMEDICAL RESEARCH SERVICE CENTER
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Luciferase (LUC) Assay Kit (Cat #: E-126)

COMPONENTS: Luciferase Assay Solution- 2 x 10 ml (200 assays), **store in aliquots shielded from light at -80ºC**
10x Cell Lysis Solution- 25 ml, store at 4°C **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

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 scintillation counters or luminometers. 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) and chloramphenicol acetyltransferase (CAT) assays. 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. Luciferase activity is best measured with a luminometer, although a scintillation counter can also be used for the measurement. Luciferase Assay Solution if stored in aliquots at -80°C is stable for many years.

PROTOCOL:

Luciferase Assay Solution- Luciferase Assay Solution should be stored in aliquots at -80°C and shielded from light after its first thawing. Note that the solution is both temperature and light sensitive.

Dilution of 10x Cell Lysis Solution- Prepare enough 1x Cell Lysis Solution by diluting the 10x Cell Lysis Solution with ice-cold dH_2O. Place the 1x Cell Lysis Solution on ice prior to use.

Preparation of Cell Extract:

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 normal phosphate buffered saline (PBS) or Hank's buffered salt solution (HBSS) twice, and aspirate off solution completely.
2. To each 35-mm dish, add 100 µl ice-cold 1x Cell Lysis Solution, and 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 maximal speed for 5 min.
3. Pipet 10 − 20 µl cell lysate (supernatant) to a luciferase assay tube. Add 100 µl Luciferase Assay Solution followed by 2 sec of gentle mixing. Immediately measure light emission using a luminometer or a scintillation counter. Background light emission is measured by using 10 − 20 µl 1x Cell Lysis Solution as blank. Subtract the background reading from sample reading. Data are typically expressed as relative light unit (RLU) normalized by a control enzyme activity or protein concentration.

Preparation of Tissue Extract:

1. Tissue sample should be washed with PBS or HBSS thoroughly to remove blood contents, which can interfere with the assay. Tissue is homogenized in 1x Cell Lysis Solution, and lysate is clarified by centrifugation. Use ~50 mg tissue per ml of 1x Cell Lysis Solution for tissue homogenization.
2. Pipet 10 − 20 µl clarified tissue lysate to a luciferase assay tube. Add 100 µl Luciferase Assay Solution followed by 2 sec of gentle mixing. Immediately measure light emission using a luminometer or a scintillation counter. Background light emission is measured by using 10 − 20 µl 1x Cell Lysis Solution as blank. Subtract the background reading from sample reading. Data are typically expressed as relative light unit (RLU) normalized by a control enzyme activity or protein concentration.

Additional information:

- Protein assay can be performed using our **Protein Assay Kit/DC (cat#: A-117)**.
- PBS is not provided in the kit, but is required for cell and tissue washing. Recipe for PBS is 2.68 mM KCl, 137 mM NaCl, 1.47 mM KH_2PO_4, & 8.06 mM Na_2HPO_4.