ab287865 – Luciferase Reporter Assay Kit

For the measurement of expressed luciferase in vitro.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287865

Storage and Stability
The entire kit may be stored at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
Substrate A	1 vial	-20°C
Substrate B	1 vial	-20°C
Cell Lysis Buffer	100 ml	-20°C

Materials Required, Not Supplied
These materials are not included in the kit, but will be required to successfully utilize this assay:

- Luminescence Microplate Reader

Reagent Preparation

- Ensure that all reagents have reached room temperature before performing assays.
- Reconstitute Substrate A and B: Add 20 ml of Cell Lysis Buffer to each vial and mix well.
- Store both substrates at -70°C after each use. Store Lysis Buffer at 4°C.

Δ Note: *The following protocol is designed for using with adherent cultures growing in 35-mm tissue culture plates. If you are using plates of different size, adjust the volume proportionally. Read entire protocol before starting experiments.*

Assay Procedure

A. Preparation of Cell Lysate: Remove media from cell culture plates and rinse once with PBS:
   1. Add 1 ml of PBS and collect cells from plates by scraping and then transfer to a 1.5 ml microcentrifuge tube. Spin cells at 5000 rpm for 3 min and remove PBS.
   2. Resuspend cells in 200 µl Cell Lysis Buffer and incubate on ice for 5 min. Centrifuge at 14000 rpm for 1 min. Transfer extract (supernatant) to a fresh tube and use immediately, or store at –70°C.

B. Luciferase Assay:
   1. Place 20 - 100 µl cell extract into an assay cuvet or microplate well. Be sure to use the same volume for each sample.
   2. Add 100 µl Substrate A.
   3. Within 10 min, inject 100 µl Substrate B. Read the signal immediately using a luminometer.

Δ Note: *The amount of extract required may vary depending on the luciferase expression level and the instrumentation used; the amount used should be adjusted to keep the signal within the linear range of the assay. The time between adding substrate B and reading signal should be as short as possible (1-2 sec) and consistent from sample to sample.*

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)