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Firefly Luciferase Assay Kit

11/20

(Catalog # K2062-200, -1000 assays; Store at -20 °C)

I. Introduction:

Luciferase-based assays are the most sensitive assays for studying gene regulation and function. Firefly luciferase is 100-fold more sensitive than the alternative reporters including chloramphenicol acetyltransferase, β-Glucuronidase and green fluorescent protein assays. **BioVision's Firefly Luciferase Assay Kit** is designed for an accurate, sensitive and efficient quantitation of firefly luciferase reporter enzyme activity for studying gene reporter regulation and function. In this assay, firefly luciferase catalyzes the oxidative carboxylation of luciferin, a reaction with the highest efficiency of any known bioluminescent system to produce light. It is designed to detect and quantitate firefly luciferase expression in mammalian cells. The kit ensures maximum sensitivity, consistency and strong bioluminescent signal.

$$\text{Luciferin} + O_2 + \text{ATP} \xrightarrow{\textbf{Luciferase}} \text{Oxyluciferin} + CO_2 + \text{PPi} + \text{Light}$$

II. Applications:

- Study promoters and its interaction with transcription factors.
- Determine the effect of inhibitors or inducers in *in vitro* cell-based assays.

III. Sample Type:

- Cell culture: adherent and suspension cells

IV. Kit Contents:

Components	K2062-200 200 assays	K2062-1000 1000 assays	Cap code	Part Number
Substrate A	1 Bottle	5 Bottles	NM	K2062-xx-1
Substrate B	20 ml	5 x 20 ml	Red/NM	K2062-xx-2
Cell Lysis Buffer	20 ml	100 ml	NM	K2062-xx-3

V. User Supplied Reagents and Equipment:

- 1X PBS
- Cell Culture media
- Tissue culture plates
- Flat-bottom, white 96-well plate
- Plate Luminometer

VI. Storage Conditions and Reagent Preparation:

Store the kit at -20 °C, protected from light. Read the entire protocol before performing the assay.

- **Luciferase Substrate working solution:** Prepare the **Luciferase Substrate working solution** by mixing the contents of 1 Bottle of Substrate A with 20 ml of Substrate B. Mix well to dissolve. Immediately, divide the Luciferase Substrate working solution into aliquots and store at -70 °C. Keep the Luciferase Substrate working solution on ice at all times. Do not thaw the Substrate working solution above 25 °C. **Note: Do not store the Luciferase Substrate working solution at 4 °C or -20 °C.**
- **Cell Lysis Buffer:** Ready to use. Store at 4 °C.

VII. Firefly Luciferase Assay Protocol:

Note:

The following protocol was optimized using adherent/suspension cells grown in a 96-well tissue culture plate. Adjust volumes accordingly for other plate formats. Assay volume is 120 µl. Growth conditions, cell numbers/well and other factors may affect results. Thus, assay optimization is required for every cell lines to be tested.

1. Sample Preparation:

Adherent cells:

1) Remove the cell media by gentle aspiration. **Note:** Do not disturb the cell layers.

2) Rinse the cells with 1X PBS and remove the PBS by gentle aspiration as much as possible.

3) Add 25 µl Cell Lysis Buffer to each well and incubate the plate on a shaker on ice for 5 min.

4) Collect the cells by scraping and transfer the cell lysate to a microcentrifuge tube. Centrifuge at 16,000 x g for 1 min at room temperature (RT) and transfer the clear cell lysate to a new tube. Discard the pellet.

Suspension cells:

1) Collect the cells into a centrifuge tube and pellet by centrifugation at 730 x g for 5 min at RT. Remove the supernatant.

2) Wash the cells by adding 200 µl 1x PBS followed by centrifugation at 730 x g for 5 min at RT.

3) Remove the PBS and add 25 µl Cell Lysis Buffer to each tube. Incubate the cells for 5 min on ice.

4) Centrifuge at 16,000 x g for 5 min at RT and transfer the clear cell lysate to a new tube.

Notes:

a. Increase the volume of Cell Lysis Buffer, if flasks are used. i.e use 0.4 ml Cell Lysis Buffer per 60 mm culture dish or 1 ml Cell Lysis Buffer per 100 mm culture dish.

b. We recommend using freshly prepared cell lysates. If not used immediately, store the cell lysates at -70°C.

c. Determine the protein concentration using any preferred Protein Quantification Method (BioVision Cat # K819).

2. Addition of Luciferase Substrate working solution: Prepare two wells of a 96-well plate labeled as **"Sample"** and **"Background Control"**. Add 20 µl of cell lysate to the **Sample** well and 20 µl of Cell Lysis Buffer to the **Background Control** well respectively. Add 100 µl Luciferase Substrate working solution to both the wells.

3. Read the plate in a microplate luminometer with a setting of 10-25 sec integration. **Note:** Samples should be read within 30 min following the addition of Luciferase Substrate working solution.

4. Subtract the reading of the Background Control well from all Sample wells. Fold-change in luciferase activity is determined by comparing the results between treated and untreated cells.

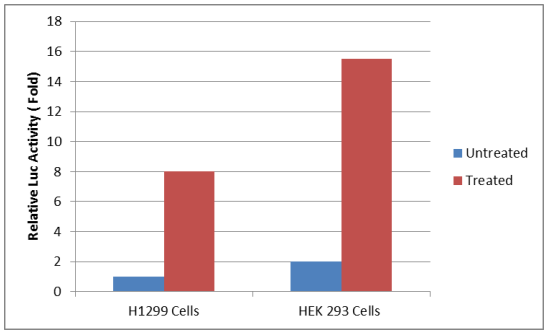

Figure. Inducible promoter-driven luciferase expression in H1299 and HEK293 cells. $1x10^5$ cells were cultured and treated with an inducible reagent for 16 hr. The cell lysates were then assayed for luciferase activity after incubation with the luciferase substrate for 1 min at RT.

VIII. Related Products:

ApoSENSOR™ Cell Viability Assay Kit (k254)
ADP/ATP Ratio Bioluminescence Assay Kit, ApoSENSOR (K255)
Bioluminescence Cytotoxicity Assay Kit (K312)
StayBrite™ Highly Stable Luciferase/Luciferin Reagent (K790)
StayBrite™ Stable ATP Bioluminescence Assay Kit (K791)
Luciferase Reporter Assay Kit (K801)
StayBrite™ Highly Stable Luciferase (7901)
D-Luciferin, Sodium Salt (7902)
D-Luciferin, Potassium Salt (7903)
D-Luciferin (Free acid) (2779)
Brite-Light™ D-Luciferin, Potassium Salt (B3000)

FOR RESEARCH USE ONLY! Not to be used on humans.