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ab228530
Luciferase Reporter Assay Substrate Kit - Firefly

For the quantification of luciferase activity in live cells and cell extracts.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Luciferase Reporter Assay Substrate Kit - Firefly (ab228530) provides a fast, simple, and homogeneous bioluminescence assay for the quantification of luciferase activity in live cells and cell extracts. This assay is based on firefly luciferase, a monomeric 61 kD enzyme that catalyzes a two-step oxidation of luciferin, which yields light at 560 nm. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format. The signal with a half-life of two to four hours provides a consistent signal across large batches of plates. The assay is compatible with the use of standard cell growth media. It has high sensitivity, and can be used for the assays that require low detection limit.

Prepare cells (samples) with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate).

Add equal volume of luciferase assay solution.

Incubate at room temperature for 10-20 minutes.

Monitor luminescence intensity.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Item	Quantity	Storage temperature (before prep)
Luciferase Sensor	1 bottle	-20°C
Assay Buffer	1 bottle (10 mL)	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well or 384-well white plates.
- Luminometer.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Luciferase assay solution

1. Thaw all the kit components to room temperature before use.
2. Transfer the whole content of Assay Buffer into the bottle of Luciferase Sensor and mix well.

   Δ Note: The reconstituted luciferase assay solution is not stable. Aliquot and store the unused reconstituted luciferase assay solution at -80 °C. Avoid freeze/thaw cycles.

6. Sample Preparation

6.1 Adherent cells:

Plate cells overnight in growth medium at 1,000 -10,000 cells/90 µL/well (96-well plate) or 250-2,000 cells/20 µL/well (384-well plate).

6.2 Non-adherent cells:

1. Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 2,000-20,000 cells/90 µL/well for a 96-well poly-D lysine plate or 500-5,000 cells/20 µL/well for a 384-well poly-D lysine plate.
2. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment.

Δ Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density. Cells may be seeded the day before or on the day of the experiment depending upon the cell type and/or the effect of the test compounds.

Δ Note: For all luminescent experiments, it is recommended to use white plates to get the best results.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Run luciferase assay

1. Treat cells (or samples) with test compounds by adding 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in desired compound buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.
   Δ Note: Typically luciferase concentrations from 1 pg/mL to 1 ng/mL are appropriate.
2. Incubate the cell plate in a 5% CO_2 incubator at 37 °C for desired period of time, typically 4 hours to overnight.
3. Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of luciferase assay solution (from Reagent preparation) and incubate the plate at room temperature for 10-20 minutes. Keep from light.
4. Monitor luminescence intensity with a luminometer.

7.2 Establish standard luciferase calibration curve:

Luciferase standard curve should be generated together with the above assay if the absolute amount of luciferase in samples needs to be calculated.

1. Make a series of dilutions of luciferase in PBS buffer with 0.1% BSA by including a sample without luciferase (as a control) for measuring background luminescence.
2. Add the same amount of the diluted luciferase solution into an empty plate (100 µL for a 96-well plate, 25 µL for a 384-well plate).
3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of luciferase assay solution (from Reagent preparation).
4. Incubate the reaction mixture at room temperature for 10-20 minutes, kept from light.
5. Record the luminescence intensity with a standard luminometer.
6. Generate the luciferase standard curve.

8. Data Analysis

The luminescence in blank wells with the growth medium is used as a control, and is subtracted from the values for the cell (or sample) wells. The background luminescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates. A Luciferase titration curve is shown in figure 1.

9. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

10. Typical Data

Data provided **for demonstration purposes only**.

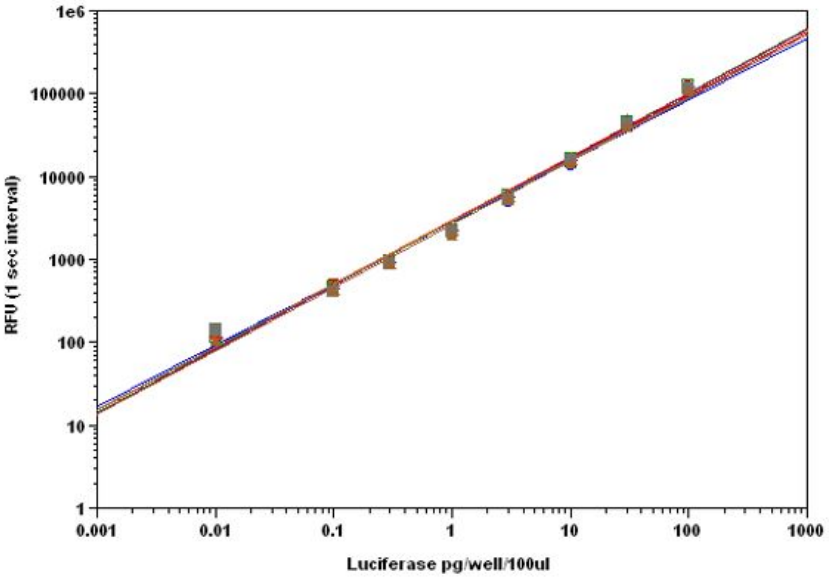

Figure 1. Luciferase dose response was measured with Luciferase Reporter Assay Substrate Kit - Firefly (ab228530) in a white 96-well plate. The kit can detect as low as 0.1 pg/well luciferase with 20 minutes to 5 hours incubation without losing signal intensity. The integration time was 1 second. The half life is more than 4 hours.

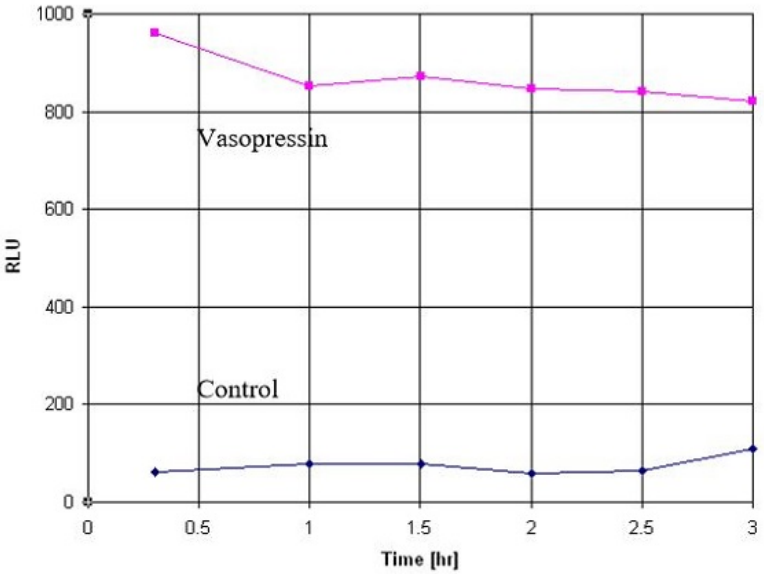

Figure 1. Reaction Kinetics of CHO-V_2R-Luc cells using Luciferase Reporter Assay Substrate Kit - Firefly (ab228530). CHO cells stably transfected with pCRE-luciferase gene and human Vasopressin receptor 2 (V2R) were plated into a white wall/clear bottom 384-well plate at 15,000 cells/well/25 µL. Cells then were treated with 100 nM of vasopressin in a 37 °C, 5% CO2 incubator for 4 hours. 25 µL of luciferase assay solution was added into the well. The kinetic data was taken every 30 minutes for up to 3 hours. The vasopressin induced luciferase signal is stable for more than 3 hours.

11. Notes

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