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ab228546
Luciferase Reporter Assay Substrate Kit - Renilla

For the measurement of luciferase activity in cells.

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

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1. Overview

Luciferase Reporter Assay Substrate Kit - Renilla (ab228546) provides a fast and sensitive method to detect the luciferase from sea pansy (*Renilla reniformis*). It uses a proprietary luminogenic formulation to quantify Renilla luciferase activity in cell-based assays. The formulation generates a luminescent product that gives strong luminescence upon interaction with Renilla luciferase. The kit provides all the essential components. It has high sensitivity and can be performed in a convenient 96-well and 384-well microtiter-plate format. The "glow-type" signal with a half-life of one hour provides a consistent signal across large number of assay plates. The assay is compatible with standard cell growth media. This kit enables the measurement of primary expression or gene expression with wild type and the synthetic hRluc genes.

Prepare cells and treat if necessary (100 µL/well/96-well plate or 25 µL/well/384-well plate).

Add equal volume of 100X luciferase assay solution.

Incubate at room temperature for 10-20 minutes.

Monitor luminescence intensity.

2. Materials Supplied and Storage

Store kit at -20C 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
100X Luciferase Substrate	1 vial	-20°C
Assay Buffer	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. Add one volume of 100X Luciferase Substrate to 100 volumes of Assay Buffer 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:

- Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 20,000-200,000 cells/90 µL/well for a 96-well poly-D lysine plate or 5000-50,000 cells/20 µL/well for a 384-well poly-D lysine plate.
- Centrifuge the plates 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.

Δ Note: It is highly recommended to use phenol red free growth medium especially DMEM and MEM which is known to absorb the light emitted from luciferases and hence quench the signal observed.

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.

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.
2. Incubate the cell plates in a 37°C, 5% CO_2 incubator for desired period of time, typically 4 hours to overnight.
3. Remove the medium completely.
4. Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of Renilla luciferase assay solution and incubate the plate at room temperature for 5-10 minutes. Keep it from light.
5. Monitor luminescence intensity with a luminometer.

8. Typical Data

Data provided **for demonstration purposes only.**

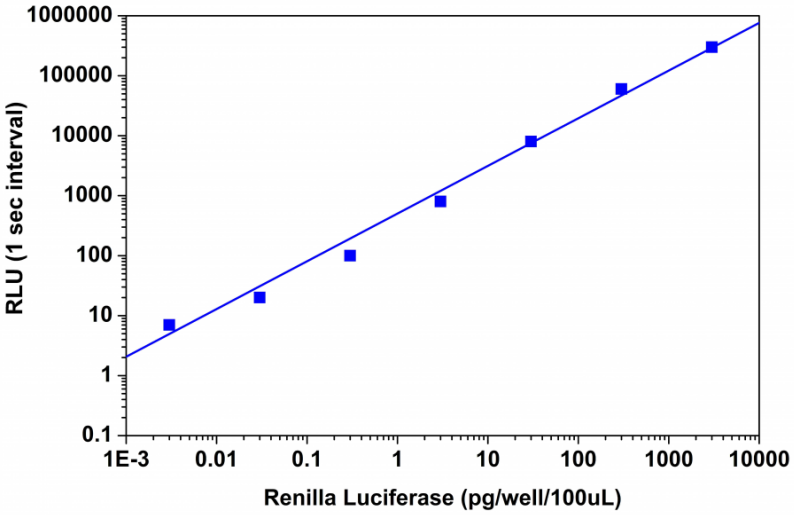

Figure 1. Renilla Luciferase was measured with Luciferase Reporter Assay Substrate Kit – Renilla (ab228546) in a white 96-well plate.

9. Notes

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