Luciferase Assay Kit

Catalogue Number: MPK003

Background: The Luciferase Assay Kit provides a sensitive, quick, and quantitative measurement of the activity of firefly luciferase produced in cultured mammalian cells. The cell extraction buffer allows efficient extraction of luciferase from many types of cultured cells within 5-10 minutes. The luciferase assay is performed by adding the luciferase substrate to the cell lysate premixed with the assay buffer. Luciferase in the cell lysate catalyzes the chemiluminescent reaction, which emits light that is readily measurable with a luminometer.

Kit Components:

Materials provided	*Quantity*
Extraction Buffer	10 ml
Assay Buffer	50 ml
Potassium Phosphate	10 ml
ATP, 100 mM	1 vial
DTT, 1 M	1 vial
Luciferin, 1mM	2 x 1 ml

Note: The Luciferase assay kit contains sufficient reagents for 100 assays performed in 24-well or 12-well tissue culture plate.

Procedures:

Preparation of Working Solution

Volume per assay	*Assay Mix*	*Substrate*
Assay Buffer	300 ul	80 ul
Potassium Phosphate	60 ul	---
ATP, 100 mM	0.4 ul	---
DTT, 1 M	4 ul	1 ul
Luciferin, 1mM	---	20 ul

Preparation of Cell Lysates

- Remove the media from the tissue culture plate wells.
- Add 100 µl Extraction Buffer into each well to cover the cells.
- Incubate the plate on shaker platform at room temperature for 5-10 minutes.
- Place the plates on ice and proceed to the next step.

Measurement of Lumino-intensity

1. Single-Tube Measurement with Manual Injection

- Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity.
- Dispense 300µl of the Luciferase Assay Mix into luminometer tubes, one tube per sample.

- Add 90µl of cell lysate to a luminometer tube containing the Luciferase Assay Mix. Mix by pipetting 2–3 times or vortex briefly.
- Add 100µl of Substrate to a luminometer tube containing the Luciferase Assay Buffer and cell lysates. Vortex briefly.
- Place the tube in the luminometer and initiate reading.
- Record the reading.

2. Single-Tube Measurement with Automatic Injection

- Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity.
- Flush the luminometer injector at least three times with Luciferase Substrate Reagent.
- Dispense 300µl of the Luciferase Assay Mix into luminometer tubes, one tube per sample.
- Add 90µl of cell lysate to a luminometer tube containing the Luciferase Assay Mix. Vortex briefly.
- Place the tube in the luminometer and initiate reading by injecting 100µl of Substrate into the tube.
- Record the reading.

3. Multiple-Tube Measurement with Automatic Injection

- Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity.
- Program the luminometer with at least three injections with Luciferase Substrate.
- Dispense 300µl of the Luciferase Assay Mix into luminometer tubes, one tube per sample.
- Add 90µl of cell lysate to a luminometer tube containing the Luciferase Assay Mix.
- Place the tubes in the luminometer chain and initiate reading by injecting 100µl of Substrate into the tube.
- Record the reading.

4. Plate-Reading Luminometers with Automatic Injection

The following protocol was designed for 96-well plate format:

- Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity.
- Dispense 300µl of the Luciferase Assay Mix into each well, one well per sample.
- Add 90µl of cell lysate to the well containing the Luciferase Assay Mix.
- Place the plate in the luminometer and initiate reading by injecting 100µl of Substrate into the well.
- The plate is advanced to the next well for a repeat of the injection and reading.
- Record the reading.