

ab308238 – Firefly Luciferase Assay Kit

For an accurate, sensitive and efficient quantitation of firefly luciferase reporter enzyme activity.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [abcam website](#)

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Substrate A (Avoid light)	1 Bottle	-
Substrate B	20 ml	-
Cell Lysis Buffer	20 ml	4 °C

Materials Required, Not Supplied

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

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

Reagent Preparation

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.

Firefly Luciferase Assay Protocol:

Δ 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 line to be tested.*

1. Sample Preparation:

Adherent cells:

- Remove the cell media by gentle aspiration.

Δ Note: Do not disturb the cell layers.

- Rinse the cells with 1X PBS and remove the PBS by gentle aspiration as much as possible.
- Add 25 µl Cell Lysis Buffer to each well and incubate the plate on a shaker on ice for 5 min.

- 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:

- Collect the cells into a centrifuge tube and pellet by centrifugation at 730 x g for 5 min at RT. Remove the supernatant.
- Wash the cells by adding 200 µl 1x PBS followed by centrifugation at 730 x g for 5 min at RT.
- Remove the PBS and add 25 µl Cell Lysis Buffer to each tube. Incubate the cells for 5 min on ice.
- Centrifuge at 16,000 x g for 5 min at RT and transfer the clear cell lysate to a new tube.

Δ Note:

- 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.
 - We recommend using freshly prepared cell lysates. If not used immediately, store the cell lysates at -70°C.
 - Determine the protein concentration using any preferred Protein Quantification Method.
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- Addition of Luciferase Substrate working solution:**
Prepare two wells of a 96-well plate labelled 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.
 - 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.
 - 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.

Technical Support

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