

Assay kits

# Luciferase Assay Kit

Detection and Quantification of Luciferase activity

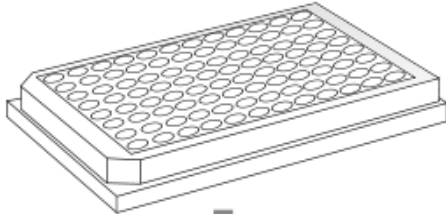
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## Protocol

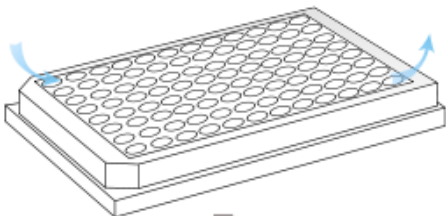
# Luciferase Assay Kit Quick Protocol

## Rapid protocol for 96-well plate

Transfect cells with plasmid or mRNA encoding Luciferase (pVectoZ-LUC or mRNA-LUC)

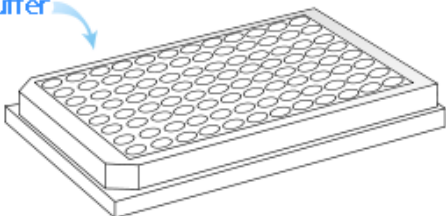


Gently remove medium from culture plate  
Rinse cells twice with PBS



Add 50  $\mu$ L per well of 1X Lysis buffer

1X Lysis Buffer  
(50  $\mu$ L/well)

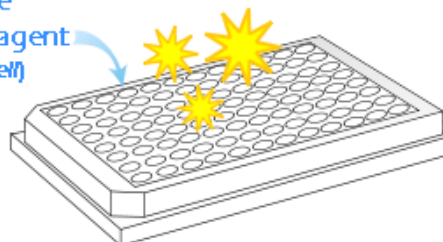


Incubate 15 min at Room Temperature



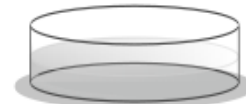
Add 100  $\mu$ L per well of Luciferase Assay Reagent

Luciferase  
Assay Reagent  
(100  $\mu$ L/well)



Within 15 min read the signal using Luminometer

## Assay Protocol



Gently remove medium from culture plate  
Rinse cells twice with PBS

Add 1X Lysis buffer

1X Lysis Buffer



Incubate 15 min at Room Temperature

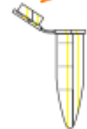
Scrape and transfer lysed cells into a microcentrifuge tube



Centrifuge: 3500 rpm x 5 min



Transfer supernatant into a new tube

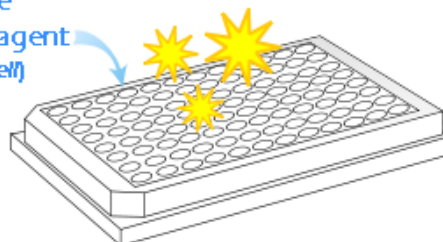


Add 50  $\mu$ L of cell lysate to an opaque 96-well plate



Add 100  $\mu$ L per well of Luciferase Assay Reagent

Luciferase  
Assay Reagent  
(100  $\mu$ L/well)



Within 15 min read the signal using Luminometer

## IMPORTANT NOTES – Before you begin

- ✓ Allow reagents to reach room temperature before beginning.
- ✓ Measurements can be performed directly into transfected well.
- ✓ Prefer using opaque 96-well/clear bottom plates for fluorescence measurement.
- ✓ Samples can be processed directly or stored at -20°C or -80°C after lysis procedure as freezing generally increases cell lysis and luciferase signal. Bring the plate to RT before assaying.
- ✓ Luciferase enzyme (not provided with this kit) can be used to generate a standard curve in cell lysis buffer

For additional information and protocols (optimization, scaling, co-transfection...) tips, troubleshooting or other applications



[www.ozbiosciences.com](http://www.ozbiosciences.com)

Any questions?



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Package content	LUC0100: 5 mL of Cell Lysis Buffer (1X) + 10 mL of Luciferase Assay Buffer + 1 vial of Luciferase Substrate lyophilised LUC1000: 50 mL of Cell Lysis Buffer (1X) + 100 mL of Luciferase Assay Buffer + 10 vials of Luciferase Substrate lyophilized
Shipping conditions	Dry ice
Storage conditions	Store the Cell Lysis Buffer at +4°C upon reception Store the Luciferase Assay Buffer and the Luciferase Substrate lyophilised at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	The luciferase assay kit is the most sensitive analytical tools for measuring gene expression.
Important notice	For research use only. Not for use in diagnostic procedures

# Protocol

The following protocol is given for measurement of Luciferase production in 96-well plate and in adherent and suspension cells.

## 1. Solution Preparation

### a. Preparation of the Luciferase Assay Reagent

- Dissolve 1 vial of lyophilized Luciferase substrate in 1 mL of Luciferase Assay Buffer. Pipette up and down and transfer it into a new tube containing 9 mL of Luciferase Assay Buffer.
- If necessary, aliquot the solution in smaller volumes to minimize freeze-thaw cycles. Store the aliquots at -20°C or -80°C and keep away from light. Equilibrate Luciferase assay reagent at room temperature before each use.

### b. Cell Lysis Buffer

This buffer is ready-to-use. Add it directly onto the cells according to the table 1

Culture plates	Lysis Buffer / per well
96 wells	50 µL
24 wells	100 µL
6 wells	500 µL

Table 1: Suggested volumes of lysis buffer required per well, depending on the type of culture plate used

## 2. Quick protocol for 96-well plate

Prepare cells in an opaque 96-well plate.

1. Gently remove medium from the cell culture plate
2. Rinse cells twice with PBS
3. Add 50µL per well of Lysis Buffer
4. Incubate the plate 15 min at room temperature
5. Optional: place the plate on an orbital shaker (150 rpm) to ensure complete cell lysis.  
**NOTE:** at this step, you can freeze and store the plate at -20°C or -80°C.
6. Add 100 µL per well of Luciferase Assay Reagent.
7. Read the signal immediately using a luminometer

## 3. Standard protocol

This protocol is suitable for any types of culture plates or dishes

Monolayer Cells

1. Gently remove medium from the cell culture plate.
2. Rinse cells twice with PBS.
3. Add 1X lysis buffer according to table 1.
4. Incubate the plate 15 min at room temperature.
5. Optional: place the plate on an orbital shaker (150 rpm) to ensure complete cell lysis.

6. Scrape the cells into a microcentrifuge tube and centrifuge at 3500 rpm for 5 min.
7. Transfer supernatant into a 96-well plate.  
**NOTE:** at this step, you can freeze and store the plate at -20°C or -80°C.
8. Add 50 µL of cell supernatant to a white opaque 96-well plate.
9. Add 100 µL Luciferase Assay Reagent to each well.
10. Read the signal immediately using Luminometer

#### Suspension Cells

1. Dispatch cells into a microcentrifuge tube and centrifuge at 950 rpm for 5 min.
2. Withdraw supernatant and resuspend the pellet into 1 X cell lysis buffer.
3. Incubate the cells 15 min at room temperature.
4. Centrifuge at 3500 rpm for 5 min.
5. Transfer supernatant into a 96-well plate.  
**NOTE:** at this step, you can freeze and store the plate at -20°C or -80°C.
6. Add 50 µL of cell supernatant to a white opaque 96-well plate.
7. Add 100 µL Luciferase Assay Reagent to each well
8. Read the signal immediately using Luminometer

# NOTES

## Additional products

- **OZBlue Cell Viability kit** for cell viability measurement
- **ROS Detection Assay Kit** for measuring Reactive Oxygen Species activity within your cells

### Purchaser Notification

#### Limited License

The purchase of the Luciferase assay kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Luciferase assay kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Luciferase assay kit reagents and documentation to OZ Biosciences, or by destroying all Luciferase assay components. Purchasers are advised to contact OZ Biosciences with the notification that a Luciferase assay kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the Luciferase assay kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

#### Product Use Limitations

Luciferase assay kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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