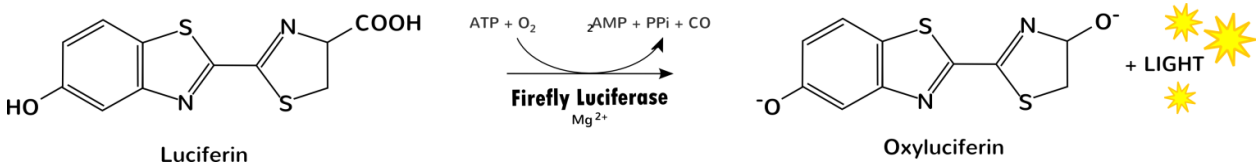
Luciferase Assay Kit

INSTRUCTION MANUAL



Luciferin

Oxyluciferin

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Luciferase Assay Kit

Instruction Manual

Fast and easy, accurate and linear cell assay kit for the detection of luciferase activity in transfected cells with the sensitivity of an enzyme-based system.

Catalog Number	Description	Content	Number of assays
LUC0100	Luciferase Assay Kit	• 1X Cell lysis Buffer (5 mL) • Luciferase Assay Buffer (10 mL) • Luciferase Substrate lyophilized (1 vial – 3mg)	100
LUC1000	Luciferase Assay Kit	• 1X Cell lysis Buffer (50 mL) • Luciferase Assay Buffer (100 mL) • Luciferase Substrate lyophilized (10 vials – 3mg)	1000

You can order this product by contacting us. For all other additional information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Technology

1.1. Description

The Luciferase Assay Kit was designed for detection and quantification of the firefly reporter enzyme from cultured cells. This kit is very sensitive due to the lack of endogenous activity in mammalian cells and provides a simple, rapid and linear measurement of luciferase expression *in vitro*.
The firefly Luciferase is first extracted from transfected cells through cell lysis and the enzyme catalyses oxidative decarboxylation of D-luciferin by oxygen into oxyluciferin using ATP|Mg^{2+} as co-substrate with emission of light (figure 1). Emitted light is finally recorded by a luminometer at 560nm.

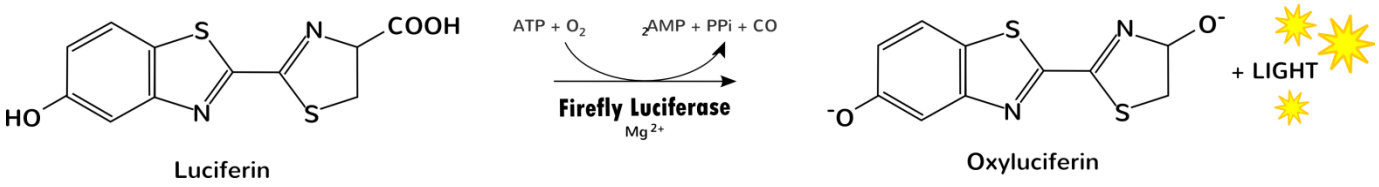


Figure 1: Firefly Luciferase catalyses the oxidation of D-Luciferin into Oxyluciferin in presence of ATP, O_2 an Mg^{2+} with emission of light.

OZ Biosciences provides all the optimized components in one kit (cell lysis buffer, luciferase substrate and luciferase assay buffer) to ensure maximal sensitivity with a consistent light output.

This kit is highly sensitive and ideal to monitor expression of pVectOZ-LUC plasmids (#PL00040, #PL00140) or mRNA Luciferase (#MRNA12-20, #MRNA12-100) transfected with OZ Biosciences transfection reagents.

- Measurements are performed directly into transfected wells.
- Cell lysis buffer is optimized to ensure maximal sensitivity
- Light emission is directly proportional to the number of luciferase enzyme molecule
- Samples can be processed directly or stored at -20°C or -80°C after lysis procedure.

1.2. Storage and kit contents

Storage — Upon receipt and for long-term use, store all reagent tubes at the indicated storage conditions (see table below). Kit components are stable for at least 1 year at the recommended storage temperature.

Shipping condition: The Luciferase assay kit is shipped with dry ice.

These kits (#LUC0100 and #LUC1000) contain sufficient reagents to perform 100 and 1000 assays respectively, in a 96-well plate format.

Components	LUC0100 100 assays	LUC1000 1000 assays	Storage
Cell Lysis Buffer (1X)	5 mL	50 mL	4°C
Luciferase Assay Buffer	10 mL	100 mL	-20°C
Luciferase Substrate (Lyophilized)	1 vial (3mg)	10 x 1 vial	-20°C

2. Applications and Protocols

2.1. Working solution preparation

A- Prepararation of the Luciferase Assay Reagent:

- Dissolve 1 vial of lyophilized Luciferase substrate in 1mL 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 below.

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

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

NOTE: Luciferase enzyme (not provided in this kit) can be used to generate a standard curve in cell lysis buffer.

2.2. Quick protocol for 96-well plates

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 50 µL 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; freezing generally increases cell lysis and luciferase signal. Bring the plate to RT before assaying

6. Add 100 µL per well of Luciferase Assay Reagent
7. Read the signal immediately using a luminometer

2.3. Standard protocol

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

Monolayer Cells

1. Remove medium from 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
    - Optional: place the plate on an orbital shaker (150 rpm) to ensure complete cell lysis
5. Scrape the cells into a microcentrifuge tube and centrifuge at 3500 rpm for 5 min
6. Transfer supernatant into a 96-well plate.
    - Optional: Freeze and store the lysed cells at -20°C or -80°C; freezing generally increases cell lysis and luciferase signal. Bring the plate to RT before performing the assay
7. Add 50 µL of cell surnageant to a white opaque 96-well plate
8. Add 100 µL Luciferase Assay Reagent to each well
9. Read the signal immediately using Luminometer

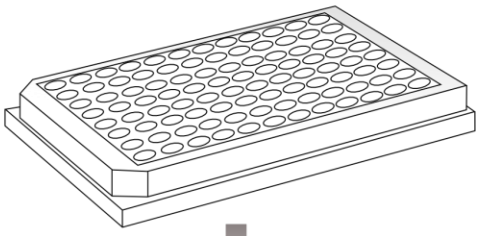
Suspension Cells

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.
    - Optional: Freeze and store the lysed cells at -20°C or -80°C; freezing generally increases cell lysis and luciferase signal. Bring the plate to RT before assaying
6. Add 50 µL of cell surnageant to a white opaque 96-well plate
7. Add 100 µL Luciferase Assay Reagent to each well
8. Read the signal immediately using Luminometer

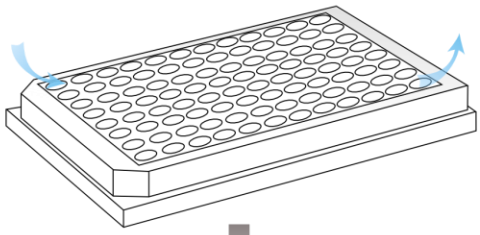
Luciferase Assay Kit - Quick Protocol

Rapid protocol for 96-well plate

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

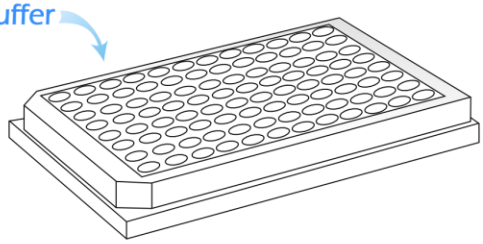


Gently remove medium from culture plate
Rince cells twice with PBS



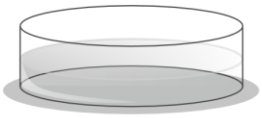
Add 50 µL per well of 1X Lysis buffer

1X Lysis Buffer
(50µL/well)



Incubate 15 min at Room Temperature

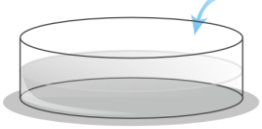
Assay Protocol



Gently remove medium from culture plate
Rince 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

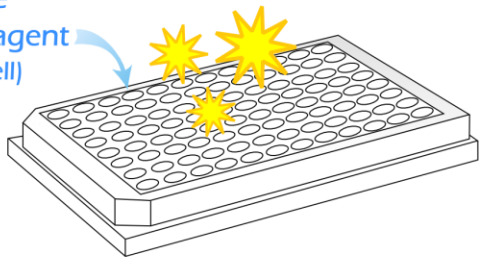
Centrifuge: 3500 rpm x 5 min

Transfer supernatant
into a new tube

Add 50 µL of cell lysate to an opaque
96-well plate

Add 100 µL per well of Luciferase Assay Reagent

Luciferase
Assay Reagent
(100µL/well)



Within 15 min read the signal using Luminometer

2.4. Performance characteristics

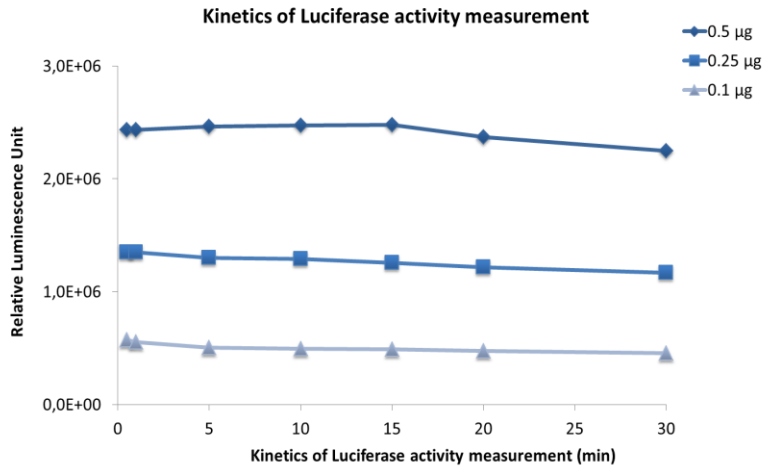


Figure 2: Kinetics of Luciferase activity measurement from 30 sec to 30 min. HEK-293 cells seeded in 6-well plates were transfected with 0.1 µg, 0.25 µg or 0.5 µg pVectOZ-LUC plasmid using DreamFect Gold. 24 H after, Luciferase activity was measured in lysed cells at several times from 30 sec to 30 min after the addition of luciferase assay reagent.

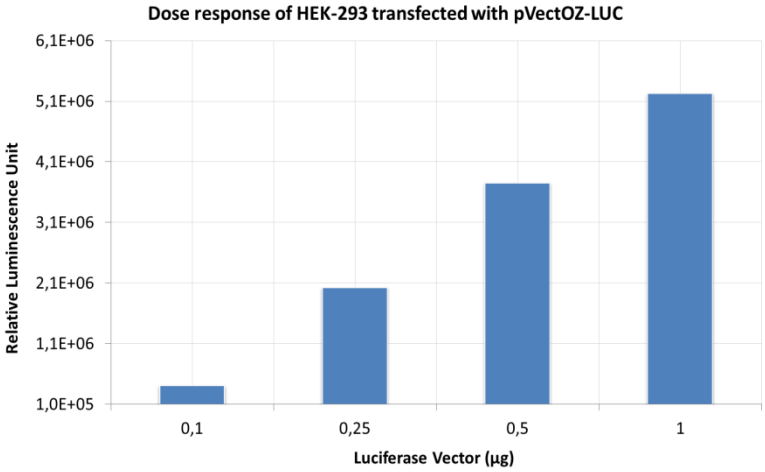


Figure 3: Luciferase activity measurement in HEK-293 cells transfected with several amounts of DNA. HEK-293 cells seeded in 6-well plates were transfected with 0.1 to 1 µg pVectOZ-LUC plasmid using DreamFect Gold. 24 H after, Luciferase activity was measured in lysed cells using the Luciferase Assay Kit 1 min after the addition of the luciferase assay reagent.

The light emission when using OZ Biosciences Luciferase assay kit is very stable at room temperature for at least 30 min.

3. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag*(for siRNA application)*
Transfection enhancer:
CombiMag*(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag*(for Adenoviruses)*
***In vivo* Magnetofection**
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
In vivo SilenceMag (magnetic nanoparticles for in vivo gene silencing)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagentfor all types of nucleic acids)*
VeroFect*(for Vero cells)*
Ecotransfect*(Economical reagent for routine transfection)*
FlyFectin*(for Insect cells)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN and Pro-DeliverIN
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
Stabilized mRNAs
Genome Editing mRNAs, Vaccine mRNAs, Reprogramming mRNAs, Reporter Gene mRNAs
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit SEAP Assay Kit X-Gal Staining Kit Senescence Kit for Stem Cells β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, $K^+$and Na^+ 1g G-418, Sulfate 1g

Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list.http://www.ozbiosciences.com

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