

Data sheet

ONPG β -Galactosidase Assay Kit (Colorimetric)

Cat. No: CA080
500 assays

Introduction

The ONPG β -Galactosidase Assay Kit is a useful tool to quickly measure the levels of active β -galactosidase expressed in cells transfected with plasmids expressing *Lac Z*. *Lac Z* is often used reporter gene in experiments transfection because the β -galactosidase is very resistant to proteolytic degradation and its activity is easily measured. β -galactosidase performs the hydrolysis of orthonitrophenyl- β -D-galactopyranoside (ONPG) to the ortho-nitrophenol (ONP). This ONP produces as a bright yellow colour that can be detected at absorbance 420 nm. The concentration of β -galactosidase is proportional to colour produced.

Advantages/Features:

The ONPG β -Galactosidase Assay Kit provides a fast, simple, and sensitive method to quantify the enzyme expression in transfected cells.

Storage

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

Shipping condition: The ONPG β -galactosidase assay kit is shipped with gel pack (4°C)

Kit Contents

Component	Amount	Store
Buffer Lysis	10 ml	RT or 4°C
10X Assay Buffer	10 ml	RT or 4°C
Stop solution	70 ml	RT or 4°C
β -mercaptoethanol	0.5 ml	RT or 4°C
ONPG substrate solution (4mg/ml)	10 ml	-20°C
β -galactosidase enzyme (0.4U/ μ l)	100 μ l	-20°C

Reagent Preparation

- Dilute 10X Assay Buffer to make 1X solutions by adding 90 ml deionized water to 10 ml of 10X solution. Unused 1X Assay Buffer may be stored at 4°C for future use.
- Add 270 μ l β -mercaptoethanol to 100 ml 1X Assay Buffer before use.

 *β -mercaptoethanol is highly toxic. Wear gloves, lab coats and other protective gear when handling.*

Quick Protocol

1. Transfect cells with a plasmid expressing *Lac Z* gene
2. Lyse the cells using the lysis buffer
3. Transfer the lysate to a microtiter plate.
4. Prepare a β -galactosidase standard curve.
5. Add the substrate and incubate at 37 °C.
6. Read absorbance at 420 nm
7. Calculate the expression levels based on a standard curve

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Assay Procedure

A. Preparation of Cell Extract (24-72 hours post-transfection)

1. Centrifuge the cells at 250 x g for 5 minutes. Aspirate the growth medium from cells. Wash cells two times with PBS. Aspirate the final wash.
2. Add 10 μ l of Lysis Buffer. Incubate at room temperature for 10-15 minutes.
To ensure complete lysis, a quick freeze/thaw cycle (freeze at -20°C and thaw at room temperature) can also be done to obtain a good lysis.

B. Assay Protocol

3. Transfer 10 μ l of cell extracts containing β -galactosidase to the corresponding well of a 96-Well Solid Plate with lid.
4. Add 10 μ l of cell extracts without β -galactosidase like blank control to its corresponding well.
5. Add 17 μ l/well of substrate solution (ONPG) and 50 μ l 1X Assay buffer (with β -mercaptoethanol) in each well. Shake 30 seconds the plate to get homogenize the reaction.
6. Cover plate and Incubate for 30 minutes at 37 $^{\circ}\text{C}$. A faint yellow color should develop.
7. Add 125 μ l stop solution to each well. Measure absorbance through spectrophotometer at 420 nm.

C. β -Galactosidase Standard Curve

1. Prepare dilutions of β -galactosidase to a standard curve, in that way extrapolate the data to graph. Dilute 10 μ l of β -galactosidase (0.4U/ μ l) in 90 μ l of lysis buffer to obtain 40 mU / μ l.
2. Make serial dilutions to get concentration of 20, 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 mU / μ l and a blank control.
3. Add 10 μ l of serial dilutions to the wells of a 96-Well Solid Plate. The final amounts of β -galactosidase are 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0 miliunits /well.
4. **Step 5-7 of Assay Protocol.**

D. Plot the Standard Curve

1. Use the blank control to eliminate the background. Measure the samples.
2. Quantify β -galactosidase expression based on a linear standard curve.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, and is not suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for the Material Safety Data Sheet of the product.