Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

DATA SHEET

Version: 02
Revision date: 05/06/2023

1. Identification

Product name **ONPGß-Galactosidase Assay Kit (Colorimetric)**
500 assays

Cat. No **CA080**

2. Description

The ONPG B-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 βgalactosidase is proportional to colour produced.

3. Composition

Item	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/µl)	100 µl	-20°C

4. Advantages/Features

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

5. Further information

Product Use Limitations

This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer

The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. **Canvax Reagents S.L.U.** shall not be held liable for any damage resulting from handling or from contact with the above product.

6. 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 4C for future use.
- Add 270 µl 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.

Canvax Reagents, S.L.U.
Luis de Mercado Street, 19
Boecillo Technological Park
47151, Boecillo
Valladolid, Spain.

Tlf: +34 983 54 85 63
info@canvaxbiotech.com

www.canvaxbiotech.com

7. 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

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 µl 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 µl of cell extracts containing β-galactosidase to the corresponding well of a 96-Well Solid Plate with lid.

4. Add 10 µl of cell extracts without β-galactosidase like blank control to its corresponding well.

5. Add 17 µl/well of substrate solution (ONPG) and 50 µl 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 °C. A faint yellow color should develop.

7. Add 125 µl 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 µl of β-galactosidase (0.4U/µl) in 90µl of lysis buffer to obtain 40 mU /µl.

2. Make serial dilutions to get concentration of 20, 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 mU /µl and a blank control.

3. Add 10 µl 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.