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DATA SHEET

Version: 02
Revision date: 05/06/2023

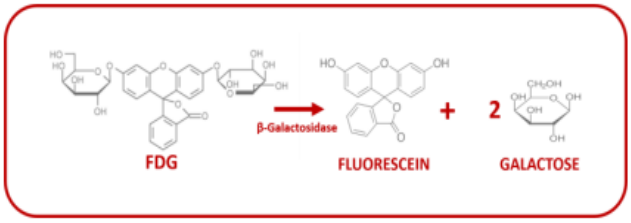
1. Identification

Product name **FDGß-Galactosidase Assay Kit**
(Fluorimetric)
500 assays

Cat. No **CA083**

2. Description

The FDG 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 **fluorescein di-βD galactopyranoside (FDG)** into a fluorescein that can be detected at excitation/emission = 490/525 nm. The concentration of β-galactosidase is proportional to fluorescence produced.



3. Composition

Component	Quantity
Reaction buffer	55 ml
Lysis Buffer	25 ml
Stop solution	25 ml
β-galactosidase enzyme (0.1mg/ml)	20 µl
β-Mercaptoethanol	500 µl
FDG solution (10 mM)	150 µl

4. Advantages/Features

- The FDG β-Galactosidase Assay Kit provides a fast, simple, and very sensitive method to quantify the enzyme expression in transfected cells.
- The kit is able to measure β-galactosidase at femtogram level.

5. Storage specifications

Upon receipt, store the kit at -20 °C. β-galactosidase substrate must be protected from light. Stop solution and Lysis Buffer can be stored at room temperature.

6. 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.

Safety Information When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.

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7. Reagent preparation

A) Preparation of Assay Buffer:

For 100 assays:
Add 30 µl of β-Mercaptoethanol to 10 ml Reaction buffer (0.3% β-Mercaptoethanol). Calculate the necessary concentration to each experiment and prepare fresh substrate solution necessary.

B) Preparation of FDG working solution:

Make FDG working solution by add 25µl of FDG solution into 5 ml of Assay Buffer.

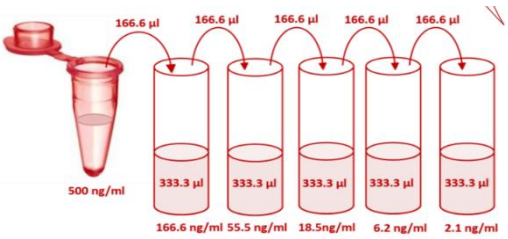
C) Preparation of Lysis Buffer working solution:

Make Lysis Buffer working solution by add 5µl of βMercaptoethanol into 5 ml of Lysis Buffer.

Assay protocol (96-Well plate)

A. β-Galactosidase Standard Curve

1. Dilute β-galactosidase to **500 ng/ml** in Lysis buffer working solution (1:200).
2. Make serial dilutions to get concentration of **166.66; 55.55; 18.52; 6.17; 2.06; 0.686** ng/ml and a blank control.
3. Add 50 µl of serial dilutions to the wells of a 96- Well Solid Plate.



B. Preparation of Cell Extract

1. **A) Adherent cells:** Aspirate the growth medium from cells. Wash cells with PBS.
B) Suspension cells: Pellet cells into a centrifuge tube and wash cells with PBS.
2. Add **50 µl/well (50 µl-2ml /tube)** of Lysis Buffer working solution. Incubate at room temperature for 10 minutes to allow cell lysis. Proceed to the Assay Protocol or freeze the sample at -80°C till use.

C. Assay Protocol

1. Transfer 50 µl of cell extracts containing β-galactosidase to the corresponding well of a 96-Well Solid Plate. ***The amount of cell extract can be adjusted depending on the level of enzyme in the sample.***
2. Add 50 µl of cell extracts without β-galactosidase (From non-transfected cells) like blank control to its corresponding well.
3. Add 50 µl/well of FDG working solution in each well. Shake 30 seconds the plate to get homogenize the reaction.
4. Incubate at 37 °C for 30 minutes -4 hours depending on the cell type. Keep away from light.
5. Add 50 µl stop solution to each well. Measure fluorescence through fluorometer at Ex/Em= 490/525 nm.

D. Plot the Standard Curve

E. Determine the Sample Concentration

1. Use the blank control to eliminate the background. Measure the samples.
2. Extrapolate the data to β-galactosidase standard curve to calculate the concentration of the enzyme in the cell extracts