

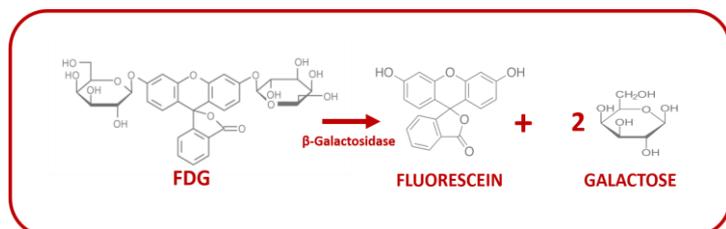
# Data sheet

## FDG $\beta$ -Galactosidase Assay Kit (Fluorimetric)

Cat. No: CA083

### Introduction

The **FDG  $\beta$ -Galactosidase Assay Kit** is a useful tool to quickly measure the levels of active  $\beta$ -galactosidase expressed in cells transfected with plasmids expressing *Lac Z*. *Lac Z* is often used reporter gene in experiments transfection because the  $\beta$ -galactosidase is very resistant to proteolytic degradation and its activity is easily measured.  $\beta$ -galactosidase performs the hydrolysis of **fluorescein di- $\beta$ -D galactopyranoside (FDG)** into a fluorescein that can be detected at excitation/emission = 490/525 nm. The concentration of  $\beta$ -galactosidase is proportional to fluorescence produced.



### Advantages/Features:

- The FDG  $\beta$ -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  $\beta$ -galactosidase at femtogram level.

### Storage

Upon receipt, store the kit at  $-20\text{ }^{\circ}\text{C}$ .  $\beta$ -galactosidase substrate must be protected from light. Stop solution and Lysis Buffer can be stored at room temperature. Kit components are stable for at least 12 months from date of receipt when stored as recommended.

### Kit Contents

Component	Amount
Reaction buffer	55 ml
Lysis Buffer	25 ml
Stop solution	25 ml
$\beta$ -galactosidase enzyme (0.1mg/ml)	20 $\mu$ l
$\beta$ -Mercaptoethanol	500 $\mu$ l
FDG solution (10 mM)	125 $\mu$ l

### Reagent preparation

#### A) Preparation of Assay Buffer:

##### For 100 assays:

Add 30  $\mu$ l of  $\beta$ -Mercaptoethanol to 10 ml Reaction buffer (0.3%  $\beta$ -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 25 $\mu$ l of FDG solution into 5 ml of Assay Buffer.

#### C) Preparation of Lysis Buffer working solution:

Make Lysis Buffer working solution by add 5 $\mu$ l of  $\beta$ -Mercaptoethanol into 5 ml of Lysis Buffer.

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Distributed by:

**Lab Unlimited**  
CARL STUART GROUP

Tallaght Business Park  
Whitestown, Dublin 24,  
Ireland  
D24 RFK3

Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: info@labunlimited.com  
Web: www.labunlimited.com

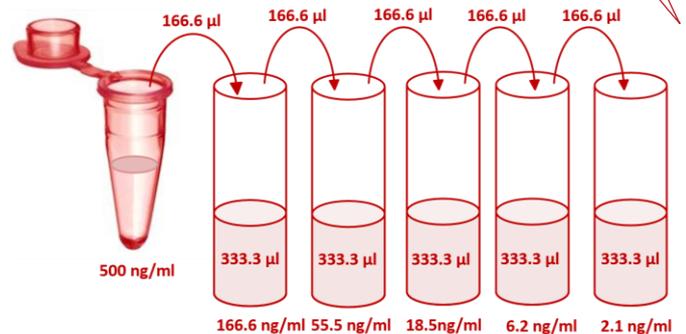
Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: info@labunlimited.co.uk  
Web: www.labunlimited.co.uk

## Assay protocol (96-well plate)

### A. $\beta$ -Galactosidase Standard Curve

1. Dilute  $\beta$ -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  $\mu$ l 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  $\mu$ l/well (50  $\mu$ l-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  $-80^{\circ}\text{C}$  till use.

### C. Assay Protocol

1. Transfer 50  $\mu$ l of cell extracts containing  $\beta$ -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  $\mu$ l of cell extracts without  $\beta$ -galactosidase (From non-transfected cells) like blank control to its corresponding well.
3. Add 50  $\mu$ l/well of FDG working solution in each well. Shake 30 seconds the plate to get homogenize the reaction.
4. Incubate at  $37^{\circ}\text{C}$  for 30 minutes -4 hours depending on the cell type. Keep away from light.
5. Add 50  $\mu$ l 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  $\beta$ -galactosidase standard curve to calculate the concentration of the enzyme in the cell extracts.

## PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. Please refer to [www.canvaxbiotech.com](http://www.canvaxbiotech.com) for the Material Safety Data Sheet of the product.

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Ireland  
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Tel: (01) 4523432  
Fax: (01) 4523967  
E-mail: [info@labunlimited.com](mailto:info@labunlimited.com)  
Web: [www.labunlimited.com](http://www.labunlimited.com)

Quatro House, Frimley Road,  
Camberley,  
United Kingdom  
GU16 7ER

Tel: 08452 30 40 30  
Fax: 08452 30 50 30  
E-mail: [info@labunlimited.co.uk](mailto:info@labunlimited.co.uk)  
Web: [www.labunlimited.co.uk](http://www.labunlimited.co.uk)