ab176721

Beta Galactosidase Detection Kit (Fluorometric)

Instructions for Use

For monitoring β-galactosidase activity in cells.

This product is for research use only and is not intended for diagnostic use.

Version: 4 Last Updated: 31 January 2019

Table of Contents

1. Overview

E. coli β-galactosidase is a 464kD tetramer. Each unit of β-galactosidase consists of five domains, the third of which is the active site. It is an essential enzyme in cells. Deficiencies of this enzyme can result in galactosialidosis or Morquio B syndrome. In *E. coli*, β-galactosidase is produced by the activation of LacZ operon. Detection of LacZ expression has become routine to the point of detection of as few as 5 copies of β-galactosidase per cell.

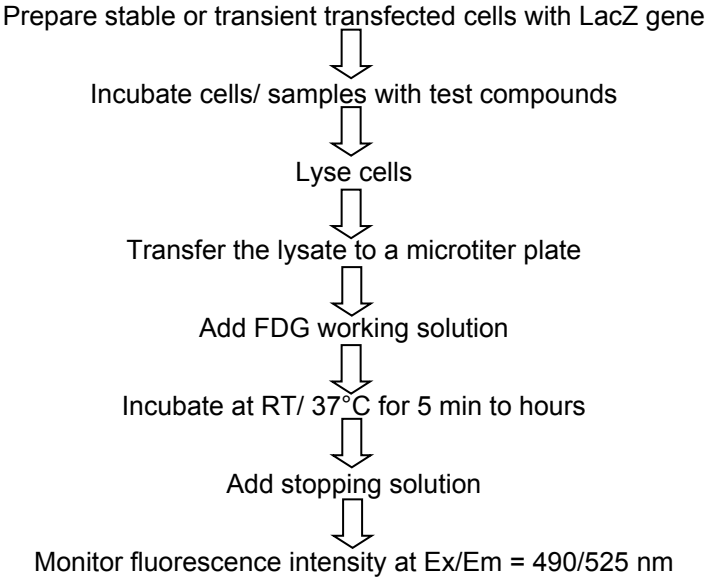
Abcam's β-galactosidase Detection Kit (Fluorometric) (ab176721) uses the fluorogenic fluorescein digalactosidease (FDG) galactosidase substrate that can sensitively distinguish LacZ+ from LacZ- cells. The non-fluorescent FDG substrate generates the strongly fluorescent fluorescein upon reaction with galactosidase. It can be used either for detecting galactosidase conjugates in ELISA type assay systems or for monitoring LacZ gene expression in cells. It can also be used for screening galactosidase inhibitors or inducers.

The galactosidase induced cleavage of FDG gives fluorescein that has spectra of Ex/Em = 490/515 nm, which can be detected with most fluorescence instruments equipped with a FITC filter set. The kit comes with all the essential components with an optimized protocol. It can be used with a fluorescence microplate reader or a fluorescence microscope.

Kit Key Features

- **Sensitive** – detect galactosidase activities in a few cells
- **Continuous** – suitable for both manual and automated operations without a mixing or separation step
- **Convenient** – formulated to have minimal hands-on-time
- **Non-radioactive** – no special requirements for waste treatment

2. Protocol Summary



3. Kit Components

Item	Quantity	Storage upon arrival	Storage after use/ reconstitution
FDG (Fluorescein di-β-D-Galactopyranoside)	1 vial	-20°C	-20°C
Reaction Buffer	50 mL	-20°C	4°C
Stop Buffer	25 mL	-20°C	4°C
Lysis Buffer	25mL	-20°C	4°C
DMSO	500 µL	-20°C	4°C
β-Mercaptoethanol	500 µL	-20°C	4°C

4. Storage and Stability

Upon arrival, store the kit at-20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

5. Materials Required, Not Supplied

- 96 or 384-well black plate with clear flat bottoms
- Multi-well spectrophotometer (ELISA reader)
- Distilled water or MilliQ
- PBS

6. Assay Protocol

1. Reagent Preparation

a) 0.3% β-mercaptoethanol assay buffer:

Add 30 µL of β-mercaptoethanol to 10 mL of Reaction Buffer, and mix well.

Note: Additional buffer is needed for preparing enzyme dilution buffer, which is used to generate a standard curve.

b) FDG:

Make FDG stock solution by adding 125 µL of DMSO into the vial of FDG. Pipette up and down to dissolve completely. Make aliquots of 25 µL (enough for 1 plate) and store any unused FDG stock solution at -20°C. Avoid repeated freeze/ thaw cycles.

Make FDG working solution by add 25 µL of FDG stock solution into 5 mL of β-mercaptoethanol assay buffer.

NOTE: DO not keep FDG solutions at room temperature for an extended period of time as spontaneous hydrolysis will occur.

NOTE: Un-used FDG solutions can be aliquoted and stored at < -20°C for more than one month. Keep from light and avoid repeated freeze-and-thaw cycles.

c) Lysis Buffer:

Make lysis buffer working solution by adding 5 µL of β-mercaptoethanol to 5 mL of Lysis Buffer before use.

NOTE: always add 0.1% β-mercaptoethanol into lysis buffer before lysing the cells.

2. Sample preparation

2.1 Adherent cells:

a) Treat cells containing LacZ gene with test compounds for a desired period of time.

b) Wash the cells twice with 1X PBS carefully so cells do not detach from the well.

c) Add lysis buffer working solution (from Step 1c) to the culture plates. Recommended volumes for various plates are listed in the following table:

Type of culture plate	**Volume of lysis buffer working solution (µL/well)**
96-well plate	50
24-well plate	250
12-well plate	500
6-well plate	1000
60 mm plate	2000
100 mm plate	4000

d) Incubate cells with the cell lysis buffer at room temperature for 10 – 15 minutes, and gently swirl the plates several times to ensure complete lysis.

e) Proceed to the FDG assay or freeze the sample at -80°C till use.

NOTE: A good lysis can be also obtained by a quick freeze- thaw cycle (freeze 1 – 2 hours at -20°C to -80°C and thaw at room temperature).

Alternatively, centrifuge the cell lysis for 2 – 3 min to pellet the insoluble material, and then assay the supernatant.

2.2 Non-adherent cells:

a) Treat cells containing LacZ gene with test compounds for a desired period of time.

b) Pellet cells carefully and wash cells twice with 1X PBS.

c) Pellet cells into a centrifuge tube, and add 50 µL – 2 mL of lysis buffer working solution (from Step 1c) to the tube. Resuspend by pipetting up and down several times.

d) Incubate the cells with the cell lysis buffer at room temperature for 10 – 15 minutes, and gently swirl the tubes several times to ensure complete lysis.

e) Proceed to the FDG assay or freeze the sample at -80°C till use.

NOTE: A good lysis can be also obtained by a quick freeze- thaw cycle (freeze 1 – 2 hours at -20°C to -80°C and thaw at room temperature).

Alternatively, centrifuge the cell lysis for 2 – 3 min to pellet the insoluble material, and then assay the supernatant.

3. Run β-galactosidase assay

a) Thaw the tube or plate of lysed cells at room temperature if needed. Perform the assay directly on the 96-well plate if the cells were seeded in a 96-well plate.

b) Add 50 µL of cell extracts (from 2d) into each well of the 96-well plate. Save some control wells for the standard curve if a standard curve is desired.

c) If a standard curve is desired: prepare a serial dilution of β-galactosidase (*E. coli*) (not provided) standards with 0.3% β –mercaptoethanol assay buffer (from Step 1a). Transfer 50 µL aliquot of each point on the standard curve to the control wells of the plate. The highest recommend amount of β-galactosidase is 200 mU (200 – 400 ng), so β-galactosidase needs to be diluted if concentration is higher than 200 mU. Follow the dilution procedure recommended in the table below for a serial dilution of standard curve:

LABEL	β-gal Standard (mU)	Assay Buffer (µL)	β-gal standard volume
A	200	990	10 µL of 20 U β-gal
B	100	200	200 µL of label A
C	50	200	200 µL of label B
D	25	200	200 µL of label C
E	12.5	200	200 µL of label D
F	6.25	200	200 µL of label E
G	3.125	200	200 µL of label F
H	1.562	200	200 µL of label G

NOTE: The dilutions for the standard curve must be prepared freshly each time the assay is performed.

Adjust standard curve to suit the specific experimental conditions, such as cell type, number, transfection efficiency, and size of the culture plates.

d) Add 50 µL of each standard and/or sample/well.

If necessary, dilute the lysates in 1X Lysis buffer when transfection efficiency is very high. Alternatively, reduce the volume of lysis buffer when transfection efficiency is low. IF the transfection is performed in a 96-well plate, or a stable cell line

was seeded into a 96-well plate, perform the assay directly on the plate.

NOTE: For endogenous β-galactosidase activity control, add 50 µL of cell lysates from non-transfected cells. For blank control, add 50 µL of 1X lysis buffer.

e) Add 50 µL of FDG working solution (from Step 1b) to each well. Incubate the plate at room temperature of 37°C for 10 min – 4 hours depending on the cell type.

f) Add 50 µL of Stop Buffer to each well. The Stop Buffer causes an increase in the fluorescence intensity of the product, in addition to terminate the reaction.

g) Measure the fluorescence intensity of the solution in each well with a fluorescence microplate reader at Ex/Em = 490/525 nm.

h) Quantify β-galactosidase expression based on a linear standard curve.

7. Data Analysis

The fluorescence in blank wells with the assay buffer and FDG working solution is used as a control, and is subtracted from the values for the cell (or sample) wells. The background fluorescence of the blank wells varies depending upon the sources of the microtiter plates. A β -galactosidase (E. coli) titration curve is shown in Figure 1.

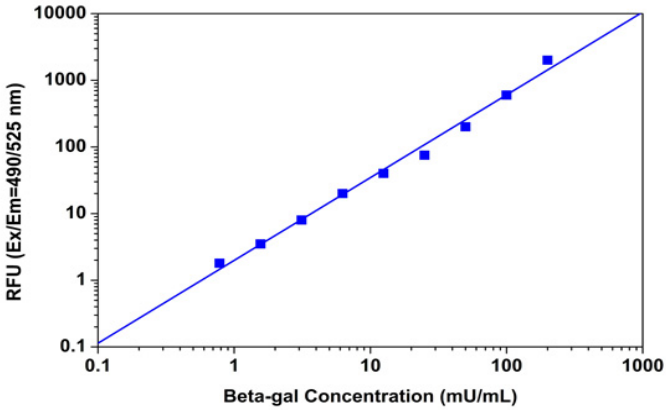


Figure 1. *β-g*alactosidase dose response was measured with B-galactosidase Detection Kit (Fluorometric) (ab176721) in a black 96-well using a fluorescence microplate reader. As low as 0.3 mU/well β-galactosidase can be detected with 30 min incubation.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

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