MANUAL

β-Galactosidase Activity Assay Kit (Colorimetric)

Catalog #: MA-BGAL

ISO 13485:2016

Introduction

β-galactosidase is one of the first and most popular reporter enzymes which are commonly used in cell biology to study the transcriptional activity of genes. β-galactosidase, encoded by the lacZ gene of E. coli, catalyzes the hydrolysis of β-galactosides into monosaccharides. It is widely used as a reporter enzyme to study gene expression, protein-protein interactions, and normalization of transfection efficiency.

β-Galactosidase Activity Assay Kit provides a simple, reproducible, and sensitive tool for measuring β-Galactosidase activity in cell lysates, plasma, serum, and other biological liquid samples. In this assay, O-nitrophenyl β-D-galactopyranoside (ONPG) is hydrolyzed into galactose and ortho-nitrophenol in the presence of β-galactosidase. Upon β-galactosidase protease cleavage, the o-nitrophenol produces a yellow color that can be detected at an absorbance of 420 nm. The optical density measured is directly proportional to the activity of β-Galactosidase present in the sample.

Storage

The entire kit should be stored below -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see the table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT
Pre-Assay Buffer	15 ml	2–8 °C, 3 months
β-Galactosidase Standard	1 vial (10 µL)	-20 °C, 3 months
Substrate	3 vials	Use immediately
β-Mercaptoethanol	1 vial (100 µL)	2–8 °C, 3 months
Stop Solution	15 ml	2–8 °C, 3 months

RT = room temperature

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 420 nm
2. Incubator at 37 °C
3. Precision pipettes to deliver 2 µl to 1 ml volumes
4. Tubes to prepare sample dilutions
5. 15 ml conical tubes

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture and wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min), making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA, or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. The sample may be assayed directly or diluted as necessary in Assay Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. The sample may be assayed directly or diluted as necessary in Assay Buffer.

NOTE: *Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.*

Reagent Preparation

REAGENT	PREPARATION
Microplate	No preparation
Pre-Assay Buffer	
β-Mercaptoethanol	
Stop Solution	
Assay Buffer	Immediately before using, add 17.5 µL β-Mercaptoethanol into 10 mL Pre-Assay Buffer to create Assay Buffer. Mix well. Prepare additional Assay Buffer as needed.
β-Galactosidase Standard	See "Standard Preparation" section.
Substrate	Quickly spin down each vial immediately before use. Reconstitute with 1 ml Assay Buffer.
Working Solution	Add 1mL reconstituted Substrate solution into 1800 µL Assay Buffer to make the **Working Solution**. This is enough to assay 38 wells. Prepare the additional vials as needed. The Working Solution should be used immediately after preparation. Keep it in the dark, on ice.

Standard Preparation

To prepare a dilution series of β-Galactosidase Standard in the concentration range of 0 U/L – 160 U/L (see table below).

1. Label 8 microtubes #1 through 8 with the following concentrations: 160, 80, 40, 20, 10, 5, 2.5, 0 U/L.
2. Pipette 196 µL Assay Buffer into labeled tube #1, and 100 µL Assay Buffer into labeled tube #2 through #8.
3. Briefly spin down the β-Galactosidase standard tube provided, and pipette 2 µL of the β-Galactosidase standard into 285.3 µL Assay Buffer to make 8000 U/L stock. Mix well.
4. Pipette 4 µL of 8000 U/L stock into tube#1, mix thoroughly, and quick spin to make a 160 U/L β-Galactosidase Standard.
5. To make the 80 U/L standard, pipette 100 µL of tube #1 into the tube labeled #2. Mix thoroughly and quick spin.
6. Repeat this step with each successive concentration, preparing a dilution series as shown in the table below. Each time, use 100 µl of the prior concentration until the 2.5 U/L is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh and used immediately.

Labeled Tubes	β-Galactosidase Standard (µL)	Assay Buffer (µL)	Standard Activity (U/L)
1	4 µL of 8000 U/L stock	196 µL	160
2	100 µL of Tube #1	100 µL	80
3	100 µL of Tube #2	100 µL	40
4	100 µL of Tube #3	100 µL	20
5	100 µL of Tube #4	100 µL	10
6	100 µL of Tube #5	100 µL	5
7	100 µL of Tube #6	100 µL	2.5
8	0µL	100 µL	0

Assay Procedure

Each β-Galactosidase Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 20 µL of the diluted β-Galactosidase Standards or samples to the 96-well microtiter plate.
2. Initiate the reaction by adding 70 µL Working Solution (See “Reagent Preparation”) to each well. Carefully shake the plate for a few seconds to mix.
3. Incubate the plate for 30 minutes at 37 °C in the dark with mixing.
4. Add 120 µL of Stop Solution to each well.
5. Measure the absorbance at 420nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 U/L) and subtract this from the absorbance values of all the other wells. These are the corrected absorbances.

Plotting the standard curves

Make a plot of corrected absorbance at 420nm as a function of β-Galactosidase activity.

Determination of sample β-Galactosidase activity

β-Galactosidase (U/L) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

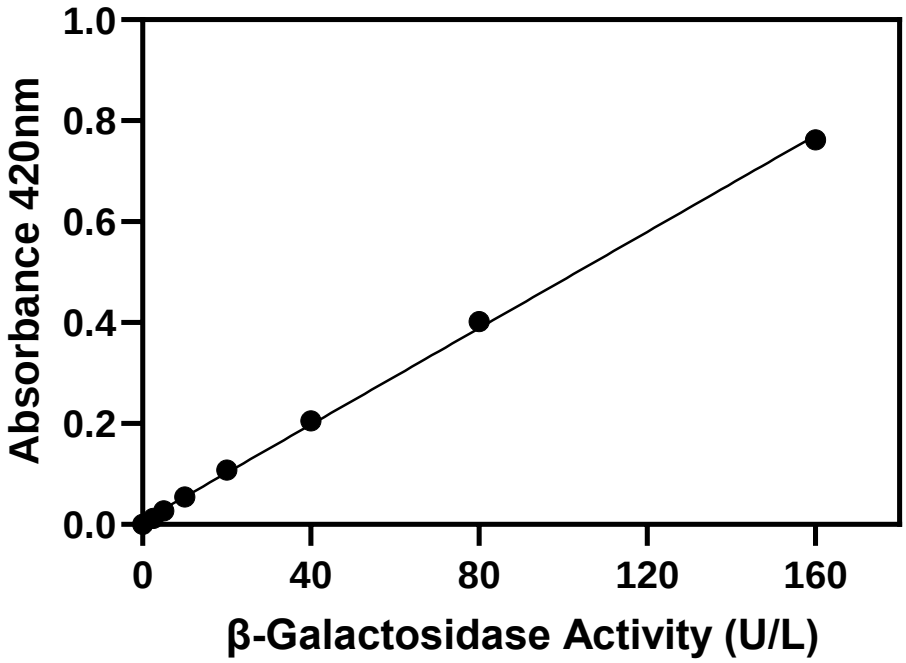
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 U/L)

Slope is from the plot of β-Galactosidase activity vs. Absorbance (as shown in Typical data below).

DF = Sample Dilution factor (DF = 1 for undiluted Samples)

Note: If the calculated β-Galactosidase activity of the sample is higher than 160 U/L, dilute the sample in assay buffer and repeat the assay.

A. Typical Data



This standard curve is for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 2.3%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 4.7%.

This product is for research use only.