SensoLyte® ONPG β -Galactosidase Assay Kit
Colorimetric

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72134**
Kit Size	500 Assays (96-well)

- ***Optimized Performance:*** This kit is optimized to detect β-galactosidase activity.
- ***Enhanced Value:*** It provides ample reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** The entire process can be completed within 30 minutes.
- ***Assured Reliability:*** Detailed protocol is provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Substrate solution	50 mL
Component B	β -galactosidase enzyme	0.1 mg/mL, 20 µL
Component C	Lysis buffer	100 mL
Component D	Stop solution	30 mL
Component E	DTT	1 M, 5 mL

Other Materials Required (but not provided)

- 96-well microplate: Clear microplates provide better signal for absorbance reading.
- Microplate reader: Capable of detecting absorbance at 420 nm.

Storage and Handling

- Store all components at -20°C.
- Keep component A away from light.
- Component D can be stored at room temperature for convenience.

Introduction

Reporter enzymes are commonly used in cell biology to study transcriptional activity of genes. β-galactosidase, is one of the first and most popular reporter enzymes. 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,[1] and normalization of transfection efficiency.[2]

The SensoLyte® ONPG β-Galactosidase Assay Kit utilizes the widely used chromogenic substrate, O-nitrophenyl β-D-galactopyranoside (ONPG) to determine the activity of β-galactosidase in transfected cells or tissues. The colorless compound 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 absorbance 420 nm. The intensity of the color produced is proportional to the concentration of β-galactosidase; thus can be used to measure β-galactosidase activity.

Protocol

1. Prepare working solutions.

Note: Warm all kit components to room temperature before starting the experiment.

1.1 Prepare cell extracts: Aspirate culture medium from cells. Wash cells by using PBS or Hanks' Balanced Salts Solution (HBSS). Add 50 µL of lysis buffer (Component C) to the microplate wells. Incubate at room temperature for 10 min to allow cell lysis.

1.2 Substrate solution: Dilute DTT (Component E) 1:10 in substrate solution (Component A). Use this DTT-containing substrate solution in all subsequent steps. Prepare fresh substrate solution for each experiment. Adjust volume as needed.

Table 1. ONPG substrate solution for one 96-well plate (100 assays)

Components	Volume
ONPG substrate solution (Component A)	45 mL
DTT (Component E)	5 mL
Total volume	50 mL

1.3 Prepare dilutions of β-galactosidase standard (optional): Dilute β-galactosidase (Component B) to 1000 ng/mL (1:100) in lysis buffer (Component C). Then make 2-fold serial dilutions to get concentration of 500, 250, 125, 62.5, 31.25, 15.63 ng/mL, include a blank control.

2. Set up the enzymatic reaction.

2.1 Add 10 µL of cell extracts containing β-galactosidase to the wells.

Note: The amount of cell extract can be adjusted depending on the level of enzyme in the samples. Use lysis buffer to dilute test samples.

2.2 Set up β-galactosidase standard (optional): Add 10 µL of serially diluted β-galactosidase reference solutions to the wells. The final amounts of β-galactosidase are 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, and 0 nanogram/well.

2.3 Simultaneously establish the following control wells, as deemed necessary:

- Negative control contains 10 µL of biological sample without β-galactosidase.

3. Detect β-galactosidase activity.

3.1 Add 90 µL/well of substrate solution into each well. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure absorbance signal: Incubate reaction for 30 min at 37°C. Optional: Add 50 µL of stop solution (Component D) to each well. Mix the reagents and measure absorbance at 420 nm.

3.3 Data Analysis:

- The absorbance reading from the blank control well is used as the background absorbance. Subtract the background reading from the readings of the other wells containing substrate.
- To evaluate the amount of β-galactosidase in the samples, use the enzyme standard curve as shown in Figure 1.

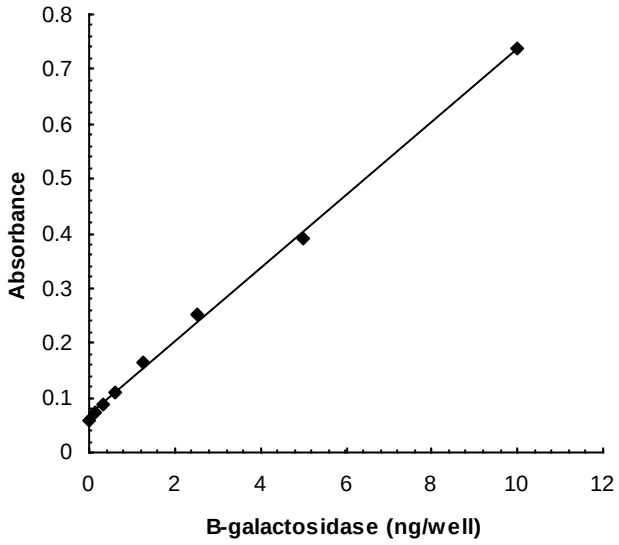

Figure 1. Detection of β-galactosidase with the SensoLyte® ONPG β-galactosidase Activity Assay Kit. The detection limit can reach as low as 0.1 ng.

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References

1. Rossi, F. et al. *PNAS* 94, 8405 (1997).
2. Thompson CD et al. *Biotechniques* 27, 824 (1999).