SensoLyte® FDG β-Galactosidase Assay Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72133**
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 min.
- ***Assured Reliability:*** Detailed protocol is provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	β-galactosidase substrate	10 mM, 250 µL
Component B	Fluorescein, reference standard	5 mM, 25 µL
Component C	β-galactosidase enzyme	0.1 mg/mL, 20 µL
Component D	Assay buffer	100 mL
Component E	DTT	1 M, 4.5 mL
Component F	Triton X-100	200 µL
Component G	Stop solution	30 mL

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom, non-binding 96-well plate.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.

Storage and Handling

- Store all components at -20°C.
- Keep components A and B away from light.
- Components F and G 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. β-galactosidase is widely used as a reporter enzyme to study gene expression, protein-protein interactions,[1] and normalization of transfection efficiency.[2]

The SensoLyte® FDG β-Galactosidase Assay Kit provides a convenient method for measuring β-galactosidase activity in transfected cells by using a fluorogenic substrate, fluorescein di-β-D galactopyranoside (FDG). In the presence of β-galactosidase, the colorless FDG is hydrolyzed into a fluorescein, that can be detected at excitation/emission = 490/520 nm. The intensity of fluorescence 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 assay buffer: Prepare fresh assay buffer for each experiment according to Table 1. **Use this DTT-containing assay buffer in all the following steps.**

Table 1. Assay buffer for one 96-well plate (100 assays).

Components	Volume
Assay buffer (Component D)	9.55 mL
1 M DTT (Component E)	450 µL
Total volume	10 mL

1.2 Prepare cell extracts: Aspirate culture medium from cells. Wash cells by using PBS or Hanks' Balanced Salts Solution (HBSS). Prepare lysis buffer by adding a final concentration of 0.1% Triton X-100 (Component F) to assay buffer. Add 50 µL of prepared lysis buffer to the microplate wells. Incubate at room temperature for 10 min to allow cell lysis.

Note: If the cell extracts are prepared in culture media, we recommend running a medium control with the β-galactosidase standard to establish the effects of the medium on assay performance.

1.3 β-galactosidase substrate solution: Dilute β-galactosidase substrate (Component A) 1:200 in assay buffer. For each experiment, prepare new substrate solution.

Table 2. β-galactosidase substrate solution for one 96-well plate (100 assays).

Components	Volume
β-galactosidase substrate (200X, Component A)	50 µL
Assay buffer	9.95 mL
Total volume	10 mL

1.4 Prepare dilutions of β-galactosidase standard (optional): Dilute β-galactosidase (Component C) to 500 ng/mL (1:200) in assay buffer containing Triton X-100. Then make 3-fold serial dilutions to get concentration of 166.66, 55.55, 18.52, 6.17, 2.06, 0.686 ng/mL, include a blank control.

2. Set up 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 assay buffer to dilute test samples.

2.2 Set up β-galactosidase standard (optional): Add 10 µL serially diluted β-galactosidase reference solutions to the wells. The final amounts of β-galactosidase are 5, 1.67, 0.56, 0.18, 0.06, 0.02, 0.007 and 0 ng/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 fluorescence signal: Incubate the reaction at 37°C for 30 min. Keep plate from direct light. Optional: Add 50 µL per well of stop solution (Component G). Mix the reagents. Measure fluorescence intensity at Ex/Em= 490/520 nm.

3.3 Data analysis:

- The fluorescence reading from the blank control well is used as the background fluorescence. Subtract the background reading from the readings of the other wells containing substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).
- To evaluate amount of β-galactosidase in the samples, use enzyme standard curve as shown in Figure 1.

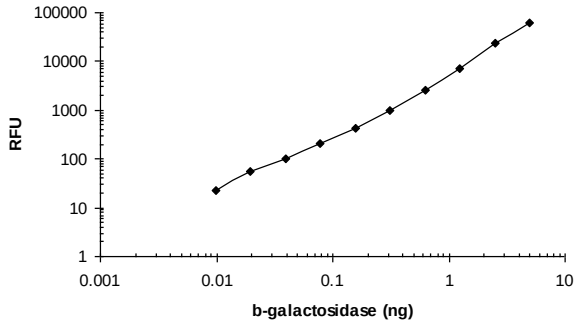

Figure 1. Detection of β-galactosidase with the SensoLyte® FDG β-Galactosidase Assay Kit. Purified β-galactosidase was serially diluted in assay buffer. The enzyme at each dilution was mixed with β-galactosidase substrate and then incubated at 37°C for 30 min. Endpoint fluorescence signal was monitored at Ex/Em=490 /520 nm (FlexStation 384II, Molecular Devices). The assay is able to detect as low as 10 pg of β-galactosidase (mean±S.D., n=3).

Appendix I: Instrument Calibration

- Fluorescein reference standard: Dilute the 5 mM reference standard (Component B) 1:20 in assay buffer containing Triton X-100 to give 250 μM stock. Perform 2-fold serial dilutions with this diluted standard to obtain 125, 62.5, 31.25, 15.6, 7.8, and 3.9 μM solutions, including an assay buffer blank. Add 10 μL/well of the serially diluted solution.
- Add 90 μL/well of the diluted β-galactosidase substrate solution (refer to 1.3).

  Note: The β-galactosidase substrate solution is added to the reference standard to normalize for the intrinsic substrate fluorescence. If multiple concentrations of substrate are used, this step must be performed for each concentration.
- Measure the fluorescence of the reference standard and substrate control wells at Ex/Em= 490/520 nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot fluorescence of reference standard as RFU (relative fluorescence unit) versus concentration.

  Note: The final concentrations of fluorescein reference standard solutions are 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0 μM. This reference standard is used to calibrate for the variation of different instruments and to account for experimental variability. Since the proteolytic cleavage of FDG includes two steps, and both the intermediate and final products have fluorescence, the FDG reference standard cannot serve as an indicator of the amount of galactosidase enzymatic reaction final product.

References

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