SensoLyte® MUG β-Galactosidase Assay Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72132**
Kit Size	500 Assays (96-well)

- ***Optimized Performance:*** This kit is optimized to detect β-galactosidase activity.
- ***Enhanced Value:*** It provides enough 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	20 mM, 250 µL
Component B	4MU, reference standard	10 mM, 50 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 460 nm with excitation at 360 nm

Storage and Handling

- Store 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, enzyme 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® MUG β-Galactosidase Assay Kit detects β-galactosidase activity in transfected cells by using a highly sensitive fluorogenic substrate, 4-methylumbelliferyl β-D-galactopyranoside (MUG). In the presence of β-galactosidase, the colorless MUG is hydrolyzed into 4-methylumbelliferone (4MU), that has a bright blue fluorescence and can be monitored at excitation/emission = 360/460 nm. The sensitivity of the assay is at femtogram level.

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)	0.450 mL
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 0.1% of 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 400 ng/mL (1:250) in assay buffer, containing Triton X-100. Then

make 5-fold serial dilutions to get concentration of 80, 16, 3.2, 0.6, 0.12, 0.024 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 4000, 800, 160, 32, 6, 1.2, 0.24, and 0 pg/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 the fluorescence intensity at Ex/Em = 360/460 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.

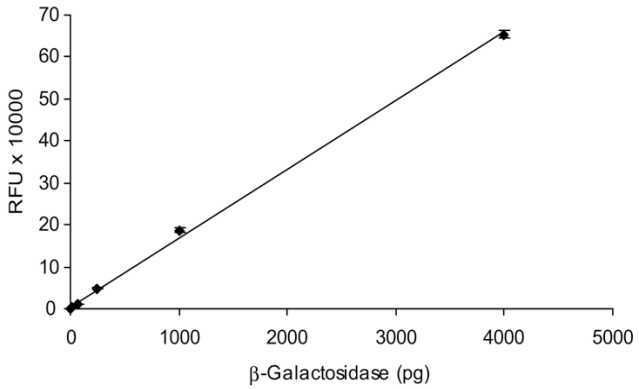

Figure 1. Detection of β-galactosidase with the SensoLyte® MUG β-Galactosidase Assay Kit. Purified β-galactosidase was serially diluted in assay buffer. The enzyme at each dilution was mixed with β-galactosidase substrate and then incubated at 37°C for 30 min. Endpoint fluorescence signal was recorded at Ex/Em=360/460 nm (FlexStation 384II, Molecular Devices). This assay is able to detect as low as 15 femtogram of β-galactosidase (mean±S.D., n=3).

Appendix: Instrument Calibration

- 4MU reference standard: Dilute the 10 mM reference standard (Component B) 1:10 in assay buffer containing Triton X-100 to give 1000 µM stock. Perform 2-fold serial dilutions with this diluted standard to obtain 500, 250, 125, 62.5, 31.25, and 15.6 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 (from step 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=360/460 nm. Use the same setting of sensitivity as used in the enzyme reaction.
- Plot 4MU fluorescence reference standard as RFU (relative fluorescence unit) versus concentration as shown in Figure 2.

  Note: The final concentration of 4MU reference standard solutions are 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0 µM. The reference standard is used to calibrate the variation of different instruments and to account for experimental variability. It is also an indicator of the amount of final product of the β-galactosidase enzymatic reaction.

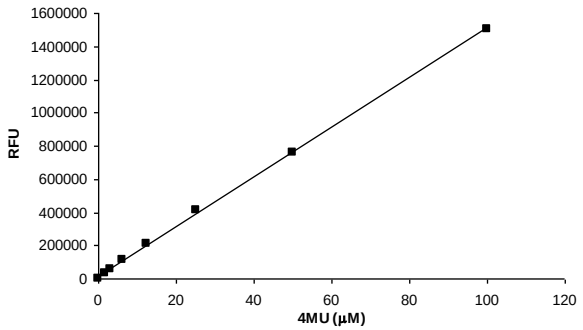

Figure 2. 4MU reference standard curve. 4MU was serially diluted in assay buffer, containing β-galactosidase substrate, and fluorescence monitored at Ex/Em= 360 nm/460 nm (FlexStation 384II, Molecular Devices).

References

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