Version 1 Last updated 5 February 2019

ab234625 β-Glucuronidase Activity Assay Kit (Fluorometric)

For the measurement of β-Glucuronidase activity in cells or animal tissues.

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

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1. Overview

β-Glucuronidase Activity Assay Kit (Fluorometric) (ab234625) provides a quick, reliable fluorometric method for measurements of β-Glucuronidase activities of samples and tissue lysates. The provided substrate, which is specific to β-Glucuronidases, is cleaved into a fluorescent product in the presence of β-Glucuronidase. This kit, when used according to the protocol, is sensitive enough to detect as little as one µU (1 pmol/min) of activity.

Prepare tissue/cell lysate samples.

Prepare 4-Methylumbelliferone Standard Curve.

Add diluted Substrate Mix to the Positive Control and test samples.

Measure fluorescence (Ex/Em = 330/450 nm) immediately after addition of substrate for 0-60 minutes at 37°C.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted β-Glucuronidase Positive Control is stable for 2 months.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
β-Glucuronidase Assay Buffer	25 mL	-20°C	-20°C
β-Glucuronidase Substrate	100 µL	-20°C	-20°C
β-Glucuronidase Positive Control	1 vial	-20°C	-80°C
4-Methylumbelliferone Standard (5 mM)	35 µL	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well black plate with flat bottom.
- Multi-well spectrophotometer (ELISA reader).
- DMSO (anhydrous).
- Dounce homogenizer (if using tissue).

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 β-Glucuronidase Assay Buffer

1. Ready to use as supplied.
2. Warm to room temperature before use.

5.2 β-Glucuronidase Substrate

1. Ready to use as supplied.
2. Warm to room temperature before use.

5.3 β-Glucuronidase Positive Control

1. Reconstitute with 55 µL of β-Glucuronidase Assay Buffer to prepare the stock solution.
2. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Use within two months.

5.4 4-Methylumbelliferone Standard (5 mM)

1. Warm 4-Methylumbelliferone Standard to room temperature before use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Prepare a 200 µM 4-Methylumbelliferone (4-MU) stock solution by adding 10 µL of 5 mM 4-MU to 240 µl β-Glucuronidase Assay Buffer. Mix well.
2. Add 0, 2, 4, 6, 8, 10 µL of 200 µM 4-MU standard into a series of wells to generate 0, 0.4, 0.8, 1.2, 1.6, and 2.0 nmol of 4-MU/well respectively. Adjust the volume of each reaction to 100 µL with β-Glucuronidase Assay Buffer.

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Tissue samples:

1. Add 100 µL ice-cold β-Glucuronidase Assay Buffer per 10 mg of sample (wet weight).
2. Homogenize well on ice using a Dounce homogenizer.
3. Centrifuge lysate at 10,000 X *g* for 5 minutes at 4°C.
4. Collect the supernatant.
5. Add 5-20 µL supernatant into a well of a black 96-well plate.
6. Adjust the volume of each reaction to 90 µL with β-Glucuronidase Assay Buffer.

7.2 Cells:

1. Resuspend cells in ice-cold Assay Buffer (10^6 cells per 100 µL) and homogenize in a dounce homogenizer.
2. Centrifuge lysate at 10,000 X *g* for 5 minutes at 4°C.
3. Collect the supernatant.
4. Add 5-20 µL supernatant into a well of a black 96-well plate.
5. Adjust the volume of each reaction to 90 µL with β-Glucuronidase Assay Buffer.

Δ Note: For unknown samples, we suggest testing several dilutions (in β-Glucuronidase Assay Buffer) to ensure the readings are within the Standard Curve range.

Δ Note: For samples exhibiting significant background, prepare parallel sample well(s) as background controls. No Substrate (Background) Control reactions are prepared by omitting the substrate mix and instead adding 5 µl β-Glucuronidase Assay Buffer.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 For Positive Control:

1. use 5 µL of the reconstituted Positive Control.
2. Adjust the volume of each reaction to 90 µL with β-Glucuronidase Assay Buffer.

   Δ Note: For suspension cells: Pellet the cells at 300 x *g* for 5 minutes at room temperature prior to media removal.

8.2 Substrate Mix:

1. Dilute stock substrate solution to working concentration by 10-fold dilution in β-Glucuronidase Assay Buffer (e.g. adding 100 µL β-Glucuronidase substrate to 900 µL β-Glucuronidase Assay Buffer; this is the Substrate Working Stock). Use Substrate Working Stock within 4 hours.
2. To initiate the reactions, add 10 µL of the Substrate Working Stock to the Positive Control and test samples.

8.3 Measurement:

1. Measure fluorescence (Ex/Em = 330/450 nm) immediately after addition of substrate for 0-60 minutes at 37°C.

   Δ Note: Incubation time depends on the β-Glucuronidase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (t_1 & t_2) in the linear range to calculate the β-Glucuronidase activity of the samples. The 4-MU Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

9. Data Analysis

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (Standard #1) from all standards, controls and sample readings. This is the corrected fluorescence.
3. If significant, subtract the sample background control from sample readings.
4. Plot the corrected values for each standard as a function of the final concentration of 4-MU.
5. Calculate the β-Glucuronidase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$.
6. Apply the ΔRFU to the 4-MU Standard Curve to get B pmol of 4-MU generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample } \beta\text{ - Glucuronidase Activity} = \frac{B}{\Delta t \times V} * D = \text{pmol/min/ml} = \mu U/mL$$

Where:

B = amount of 4-MU in the sample well calculated from standard curve in pmol.

Δt = reaction time (min.)

V = sample volume added in the sample wells (mL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

Unit Definition: 1 Unit is the amount of β-Glucuronidase that can cleave 1 µmol of substrate/min under the assay conditions at 37°C.

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided **for demonstration purposes only.**

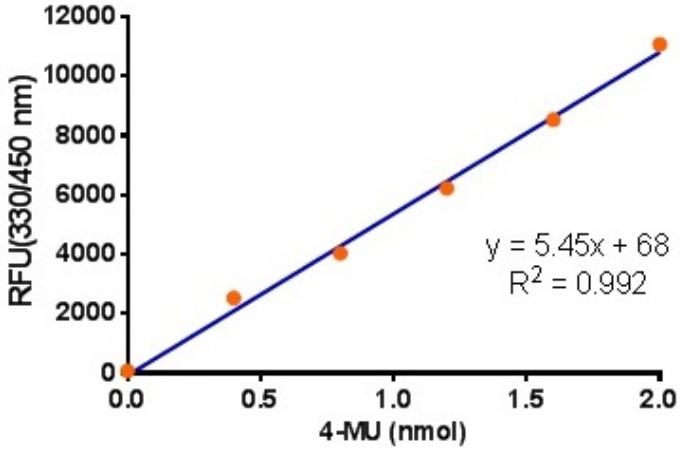

Figure 1. 4-MU standard curve.

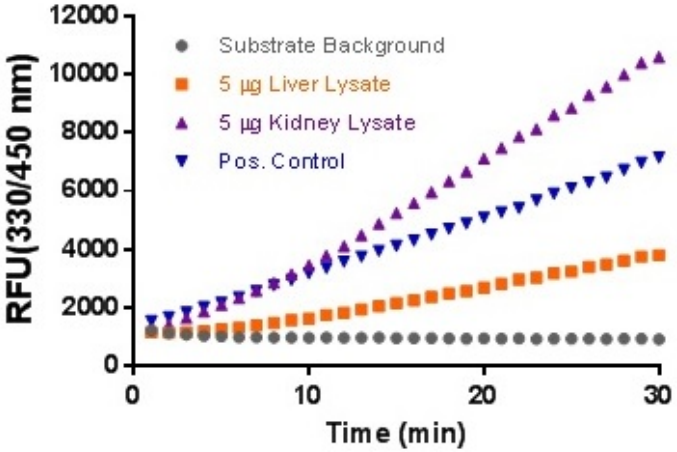

Figure 2. Time course using 5 µL positive control β-Glucuronidase and lysate samples as described.

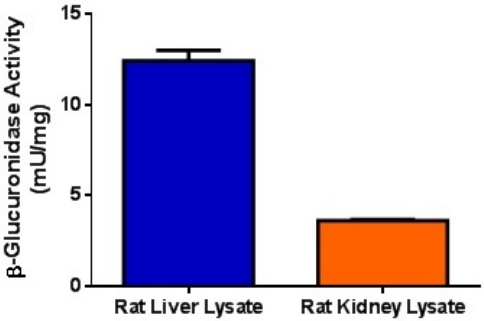

Figure 3. The β-Glucuronidase activities for Rat Kidney and Liver lysates (5 µg each), in mU/mg, were determined to be 3.61 and 12.44 mU per mg protein in sample, respectively. Assays were performed following the kit protocol.

12. Notes

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