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β-Glucuronidase Activity Assay Kit (Fluorometric)

6/16

(Catalog # K514-100; 100 assays; Store at -20°C)

I. Introduction:

β-Glucuronidases are hydrolytic enzymes responsible for the breakdown of carbohydrates. Specifically, β-Glucuronidases cleave the terminal β-D-glucuronic acid residue from the non-reducing terminus of a mucopolysaccharide chain. In humans, these enzymes are found in the lysosome of many tissue types. Loss of β-Glucuronidase activity results in a metabolic disease known as Sly syndrome. One pharmaceutical application for these enzymes is the metabolism of glucuronidated prodrugs into active pharmacological compounds. As expression and activities of β-Glucuronidases vary substantially between tissue types and disease states, these enzymes have been used to achieve targeted activation of oncotherapeutic compounds, some of which may be toxic to healthy cells not associated with malignancy or disease. It is thus important to have knowledge of the β-Glucuronidase activity in the tested sample to determine whether the prodrug or active form will predominate. BioVision's β-Glucuronidase Activity Assay Kit 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 pmole/min) of activity.

$$\text{Fl-Substrate} \xrightarrow{\beta\text{-Glucuronidase}} \text{Cleaved Substrate + Fl (Ex/Em 330/450 nm)}$$

II. Applications:

- Determination of β-Glucuronidase activity in samples
- Characterization of purified β-Glucuronidase preparations

III. Sample Type:

- Animal tissues: kidney, liver, muscle, etc.
- Purified native or recombinant protein

IV. Kit Contents:

Components	K514-100	Cap Code	Part Number
β-Glucuronidase Assay Buffer	25 ml	NM	K514-100-1
β -Glucuronidase Substrate	100 µl	Red	K514-100-2
β -Glucuronidase Positive Control	1 vial	Orange	K514-100-3
4-Methylumbelliferone Standard (5 mM)	35 µl	Yellow	K514-100-4

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- DMSO (anhydrous)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **β-Glucuronidase Assay Buffer**: Ready to use. Warm to room temperature before use. Store at -20°C.
- **β-Glucuronidase Substrate:** Ready to use. Warm to room temperature before use. Store at -20°C.
- **β-Glucuronidase Positive Control**: Reconstitute with 55 µl of β-Glucuronidase Assay Buffer to prepare the stock solution. Aliquot & store at -80°C. Avoid repeated freeze/thaw. Use within two months.
- **4-Methylumbelliferone Standard**: Warm 4-Methylumbelliferone Standard to room temperature before use. Store at -20°C. Use within six months.

VII. β-Glucuronidase Activity Assay Protocol:

1. **Sample Preparation:** For tissue samples, add 100 µl ice-cold β-Glucuronidase Assay Buffer per 10 mg of sample (wet weight). Homogenize well on ice using a Dounce homogenizer (Cat. #1998). To prepare cell lysate, resuspend cells in ice-cold Assay Buffer (10^6 cells per 100 µl) and homogenize in a dounce homogenizer. Centrifuge lysate (tissue or cell) at 10,000 X g for 5 min. at 4°C. Collect the supernatant. Add 5-20 µl supernatant into a well of a black 96-well plate. For the positive control reaction, use 5 µl of the reconstituted Positive Control. Adjust the volume of each reaction to 90 µl with β-Glucuronidase Assay Buffer.

   Notes:

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

   a. 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.

2. **4-Methylumbelliferone Standard Curve Preparation:** 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. 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.

3. **Substrate Mix:** 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.

   To initiate the reactions, add 10 µl of the Substrate Working Stock to the Positive Control and test samples.

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

   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).

5. **Calculation:** Subtract the 0 Standard reading from all readings. Plot the Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the β-Glucuronidase activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the 4-MU Standard Curve to get B pmol of 4-MU generated during the reaction time ($\Delta t = t_2 - t_1$).

Sample β-Glucuronidase Activity = B/(Δt X V) x D = pmol/min/ml x = µU/ml

Where: **B** = 4-MU amount from Standard Curve (pmol)
Δt = reaction time (min.)
V = sample volume added into the reaction well (ml)
D = Dilution Factor

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

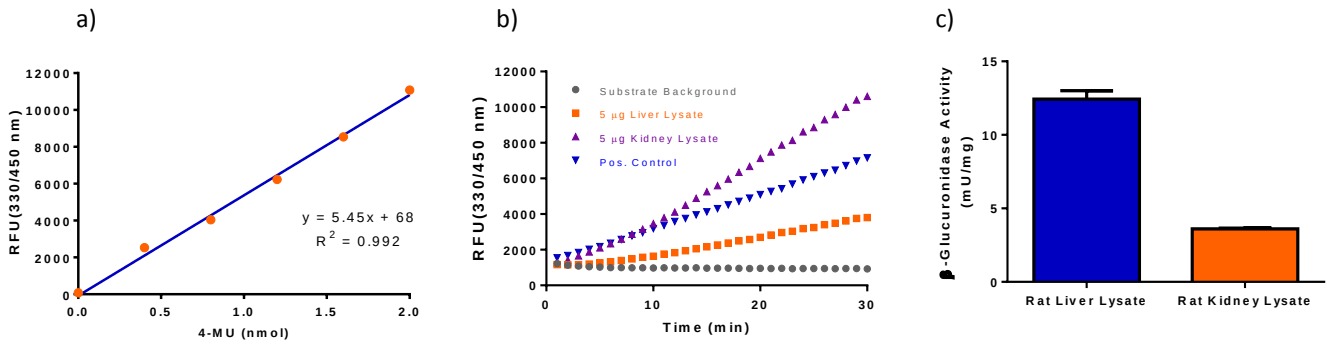

Figure: (a) 4-MU standard curve; (b) Time course using 5 µl positive control β-Glucuronidase and lysate samples as described; (c) The β-Glucuronidase activities for Rat Kidney and Liver lysates (5 µg each), in mU/mg, were determined to be 3.61 and 12.44 mU per mg protein in sample, respectively. Assays were performed following the kit protocol.

VIII. RELATED PRODUCTS:

Cytochrome P450 1A2 Activity Assay Kit (K893)
Cytochrome P450 2C19 Activity Assay Kit (K248)
Cytochrome P450 2C9 Activity Assay Kit (K895)
Cytochrome P450 3A4 Activity Assay Kit (K701)
Cytochrome P450 2D6 Activity Assay Kit (K703)
UGT Activity Assay/Ligand Screening Kit (Fluorometric) (K692)
EZSolution™ Chloramphenicol, Sterile-Filtered (2500)
Microsome Isolation Kit (K249)
Bilirubin (Total and Direct) Colorimetric Assay Kit (K716)
Valproic Acid, Sodium Salt (1647)

FOR RESEARCH USE ONLY! Not to be used on humans.