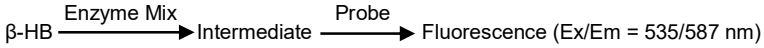
β-Hydroxybutyrate Fluorometric Assay Kit (#BN00875)

(Catalog #BN00875; 100 assays; Store at -20°C)

I. Introduction:

β-Hydroxybutyrate (β-HB) is the most abundant of the ketone bodies (~78% of total ketone bodies in blood). Ketoacidosis (high concentrations of the ketone bodies) can be caused by a variety of conditions, such as diabetes, alcoholism and severe starvation. Ketoacidosis can be fatal if not treated. Additionally, ketogenic diets can increase the concentration of ketone bodies slightly in healthy individuals. Assay Genie's β-Hydroxybutyrate Assay Kit offers simplicity, enhanced sensitivity, and can be adapted to high-throughput applications. The assay is based on enzymatic oxidation of β-HB that results in generation of fluorescent signal (Ex/Em = 535/587 nm) which is directly proportional to the amount of β-HB. The kit offers an excellent alternative for measurement of β-HB when biological sample quantities are limited or subketogenic levels are suspected. The assay can detect β-Hydroxybutyrate as low as 4 μM in a variety of biological samples.



II. Application:

- Measurement of β-Hydroxybutyrate in various biological samples
- Analysis of β-Hydroxybutyrate in pathological conditions

III. Sample Type:

- Serum, plasma
- Urine or other body fluids
- Food such as milk etc.

IV. Kit Contents:

Components	BN00875	Cap Code	Part Number
β-HB Assay Buffer	25 ml	WM	BN00875-1
GenieProbe (in DMSO)	400 µl	Blue	BN00875-2
β-HB Enzyme Mix (Lyophilized)	1 vial	Green	BN00875-3
β-HB Substrate Mix (Lyophilized)	1 vial	Red	BN00875-4
β-HB Standard (Lyophilized)	1 vial	Yellow	BN00875-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White or black plates are preferred for this assay.
- Multi-well spectrophotometer

VI. Storage and Handling:

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

VII. Reagent Preparation and Storage Conditions:

- **β-HB Assay Buffer**: Warm Assay Buffer to room temperature before use.
- **GenieProbe:** GenieProbe is Light sensitive. Warm to room temperature before use. Aliquot & store at -20°C. Stable for two months.
- **β-HB Enzyme Mix:** Reconstitute with 220 µl β-HB Assay Buffer. Pipette gently to dissolve. Aliquot & store at -20°C. Keep on ice while in use. Stable for two months.
- **β-HB Substrate Mix:** Reconstitute with 220 µl β-HB Assay Buffer. Mix well. Aliquot & store at -20°C. Protect from light. Stable for two months.
- **β-HB Standard:** Reconstitute with 100 µl ddH_2O to generate 10 mM solution. Store at -20°C. Stable for two months.

VIII. β-Hydroxybutyrate Assay Protocol:

1. **Sample Preparation:** Samples should be deproteinized using a 10 kDa Spin Column. Briefly, add sample to the spin column, centrifuge at 10,000 x g for 10 min. at 4°C. Collect the filtrate. Add 2-25 µl of filtrate into desired well(s) in 96-well plate. Adjust the volume to 50 µl/well with β-HB Assay Buffer.

   Notes:

   a. β-HB concentrations can vary over a wide range. In serum, normal range is 0.02 - 0.4 mM that can exceed up to 3 mM in diabetic ketoacidosis & up to 47 mM in alcoholic ketoacidosis. For unknown samples, we recommend to test several doses to ensure the readings are within the Standard Curve range.

   b. For samples having high background, prepare parallel sample well(s) as background control(s).

   c. Endogenous compounds may interfere with the assay. To ensure accurate determination of β-HB in the test samples or for samples having low concentration of β-HB, we recommend spiking samples with a known amount of β-HB Standard (400 pmol).

2. **Standard Curve Preparation:** Dilute the β-HB Standard to 0.1 mM by adding 10 µl of 10 mM β-HB Standard to 990 µl of ddH_2O. Mix well. Add 0, 1, 2, 3, 4 & 5 µl of diluted Standard into series of wells in a 96-well plate to generate 0, 100, 200, 300, 400 & 500 pmol/well of β-HB Standard. Adjust the volume to 50 µl/well with β-HB Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	* Background Control Mix
β-HB Assay Buffer	42 µl	44 µl
β-HB Enzyme Mix	2 µl	---
β-HB Substrate Mix	2 µl	2 µl
β-HB	4 µl	4 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing Standards and samples. Mix.

* For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix.

4. **Measurement:** Incubate the plate at room temperature for 30 min., protected from light. Measure fluorescence (Ex/Em = 535/587 nm) in a microplate reader.
5. **Calculation:** Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the β-HB Standard Curve. For unspiked samples, apply the corrected OD to the β-HB Standard Curve to get B nmol of β-HB in the sample well.

Sample β-HB concentration (C) = B/V X D pmol/µl = nmol/ml or µM

Where: **B** is the amount of β-HB in the sample well (pmol)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

For spiked samples, β-HB amount in sample well (B) = $\left(\frac{(\mathrm{OD}_{\text{sample (corrected)}})}{(\mathrm{OD}_{\text{sample + β-HB Std(corrected)}})\text{-}(\mathrm{OD}_{\text{sample(corrected)}})}\right) * \beta\text{–}HB\ Spike\ (\mathrm{pmol})$

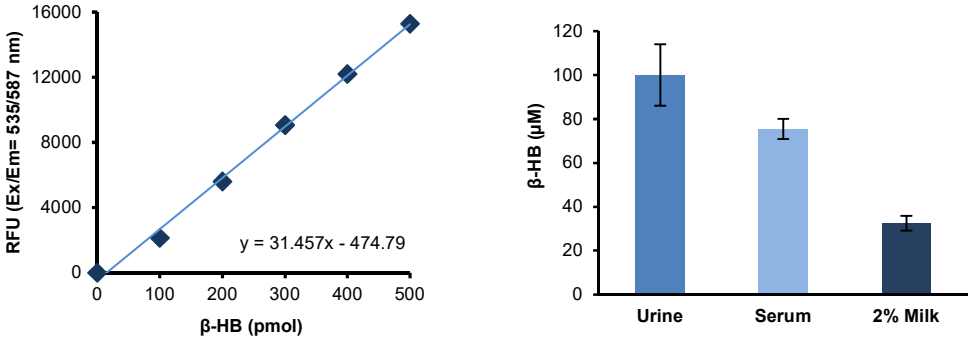


Figure: a) β-Hydroxybutyrate Standard Curve. b) Measurement of β-Hydroxybutyrate concentration in human urine (2.5 µl), serum (2.5 µl) & 2% Milk (5 µl). All samples were deproteinized using 10 kDa Spin Column & spiked with known amount of β-Hydroxybutyrate (400 pmol). Assays were performed following the kit protocol.

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