Colorimetric β-Hydroxybutyrate (Ketone Body) Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13830 (200 tests)	Keep in freezer and protect from light	Absorbance microplate readers

Introduction

Ketone bodies are produced by the liver and used peripherally as an energy source when blood glucose levels drop. The two main ketone bodies are β-hydroxybutyrate (β-HB) and acetoacetate (AcAc), while acetone is the third abundant ketone body. Normally these two predominant ketone bodies are present in small amounts in the blood during fasting (low food intake) and prolonged exercise. In patients who have diabetes, alcohol or salicylate poisoning, hormone deficiency, childhood hypoglycemia and other acute disease states, large quantities of ketone bodies are found in the blood. The over-production and accumulation of ketone bodies in the blood (ketosis) can lead to pathological metabolic acidosis (ketoacidosis). In extreme cases, ketoacidosis can be fatal. Blood ketone testing methods that quantify β-HB, the predominant ketone body in the blood (approximately 75%) have been used for diagnosing and monitoring treatment of ketoacidosis. AAT Bioquest's Colorimetric β-Hydroxybutyrate Assay Kit offers a sensitive fluorescent assay for measuring β-HB levels in biological samples. This assay is based on an enzyme coupled reaction of β-HB, in which the product NADH can be specifically monitored by a fluorescent NADH sensor. The fluorescence signal can be measured by an absorbance microplate reader with the OD ratio at the wavelength of 570 nm to 610 nm. With this Colorimetric β-hydroxybutyrate Assay Kit, we were able to detect as low as 4 µM β-HB in a 100 µL reaction volume.

Kit Components

Components	**Amount**
Component A: Enzyme Mix	2 bottles (lyophilized powder)
Component B: Assay Buffer	1 bottle (10 mL)
Component C: NAD	1 vial
Component D: β-Hydroxybutyrate (β-HB) Standard	1 vial (10 uL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare β-HB assay mixture (50 µL) → Add β-HB standards or test samples (50 µL) → Incubate at room temperature for 10-30 min → Monitor Absorbance increase at OD ratio of 570/610 nm

Note: Thaw one vial of each kit component at room temperature before starting the experiment.

1. Prepare NAD stock solution (100×):

Add 100 µL of H_2O into the vial of NAD (**Component C**) to make 100× NAD stock solutions.

2. Prepare β-HB standard stock solution:

Add 1 mL of H_2O or 1×PBS buffer into the vial of β-HB standard (**Component D**) to make 100 mM β-HB standard stock solution.

Note: The unused β-HB *standard stock solution should be divided into single use aliquots and stored at -20 °C.*

3. Prepare serial dilutions of β-HB standard:

3.1 Add 10 µL of β-HB standard stock solution (100 mM, from Step 2) into 990 µL 1×PBS buffer to generate 1 mM β-HB standard solution.

Note: Diluted β-HB *standard solution is unstable, and should be used within 4 hours.*

3.2 Take 200 µL of 1 mM β-HB standard solution to perform 1:3 serial dilutions in PBS to get approximately 300, 100, 30, 10, 3, 1 and 0 µM serial dilutions of β-HB standard.

3.3 Add serial dilutions of β-HB standard and β-HB containing test samples into a clear bottom 96-well microplate as described in Tables 1 and 2.

Table 1 Layout of β-HB standards and test samples in a clear bottom 96-well microplate

BL	BL	TS	TS	….	….						
HB 1	HB 1	….	….	….	….						
HB 2	HB 2										

HB 3	HB 3										
HB 4	HB 4										
HB 5	HB 5										
HB 6	HB 6										
HB 7	HB 7										

Note: HB= β-HB Standards, BL=Blank Control, TS=Test Samples.

Table 2 Reagent composition for each well

β-HB Standard	**Blank Control**	**Test Sample**
Serial Dilutions*: 50 µL	1×PBS Buffer : 50 µL	50 µL

**Note: Add the serially diluted β-HB standards from approximately 7 µM to 0.5 mM into wells from HB1 to HB7 in duplicate.*

4. Prepare β-HB assay mixture:

4.1 Add 5 mL of Assay Buffer (**Component B**) into one bottle of Enzyme Mix (**Component A**).

4.2 Add 50 µL NAD stock solution (**Component C**, from Step 1) into the bottle of **Component A+B** (from Step 4.1), and mix well to make β-HB assay mixture (**Component A+B+C).**
Note: This β-HB assay mixture is enough for one 96-well plate. The unused β-HB assay mixture should be divided into single use aliquots and stored at -20 °C.

5. Run β-HB assay:

5.1 Add 50 µL of β-HB assay mixture (from Step 4.2) to each well of β-HB standard, blank control, and test samples (see Step 3.3) to make the total volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of β-HB assay mixture into each well.

5.2 Incubate the reaction at room temperature for 10-30 minutes, protected from light.

5.3 Monitor the absorbance increase with an absorbance plate reader at OD ratio of 570/610 nm.

Data Analysis

The absorbance reading in blank wells (with PBS and β-HB assay mixture only) is used as a control, and is subtracted from the values of those wells with the β-HB standards and test samples. A β-HB standard curve is shown in Figure 1. Calculate the β-HB concentrations of the samples according to the β-HB standard curve.

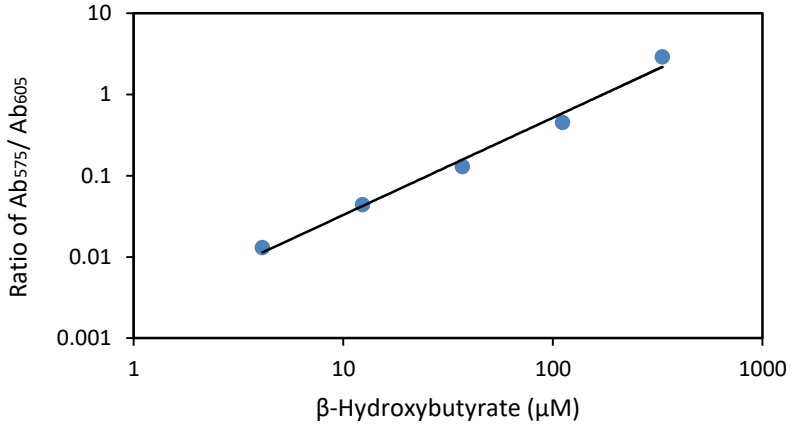

Figure1. β-Hydroxybutyrate (β-HB) dose response was measured with the Colorimetric β-Hydroxybutyrate Assay Kit on a black wall/clear bottom 96-well plate using a SpectraMax microplate reader (Molecular Devices). As low as 4 µM β-HB can be detected with 30 min incubation.

References

1. Laffel L. (1999) Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev.
2. Nosadini R, Avogaro A, Doria A, Fioretto P, Trevisan R, Morocutti A. (1989) Ketone body metabolism: a physiological and clinical overview. Diabetes Metab Rev.
3. Mitchell GA, Kassovska-Bratinova S, Boukaftane Y, Robert MF, Wang SP, Ashmarina L, Lambert M, Lapierre P, Potier E. (1995) Medical aspects of ketone body metabolism. Clin Invest Med.
4. Arlan LR. (1996) Diabetic Ketoacidosis (DKA): Treatment Guidelines. Clin Pediatr.