EnzyChrom™ Ketone Body Assay Kit (EKBD-100)

Quantitative Colorimetric Determination of Ketone Body at 340nm

DESCRIPTION

KETONE BODIES (acetoacetic acid and 3-hydroxybutyric acid) are produced in the liver mainly from oxidation of fatty acids, and are normally present at low concentrations in urine and blood. Increased ketone concentrations in the blood may lead to metabolic acidosis, which has been associated with diabetes, childhood hypo-glycaemia, growth hormone deficiency, alcohol or salicylate intoxication and inborn errors of metabolism.

Simple, direct and automation-ready procedures for measuring acetoacetic acid (AcAc) and 3-hydroxybutyric acid (BOH) are very desirable. BioAssay Systems' EnzyChrom™ ketone body assay is based on 3-hydroxybutyrate dehydrogenase catalyzed reactions, in which the change in NADH absorbance, measured at 340nm, is directly related to the AcAc and BOH concentrations,

$$\text{AcAc} + \text{NADH} + \text{H}^+ \underset{pH\ 9.5}{\overset{pH\ 7.0}{\rightleftharpoons}} \text{BOH} + \text{NAD}^+$$

APPLICATIONS

Direct assays of ketone body in serum, plasma, urine and other biological samples.

KEY FEATURES

Sensitive and accurate. Uses 10 µL sample. Linear detection range of 0.12 to 8 mM for each ketone body in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the optical density at room temperature.

High-throughput. Can be automated as a high-throughput 96-well plate assay for many samples per day.

KIT CONTENTS (200 TESTS IN 96-WELL PLATES)

AcAc Buffer:	20 mL	**BOH Buffer**:	20 mL
AcAc Reagent:	Dried	**BOH Reagent**:	1 mL
AcAc Standard:	200 µL	**BOH Standard:**	200 µL
HBDH Enzyme:	120 µL		

Storage conditions. The kit is shipped on ice. Store all reagents at -20°C. Shelf life: 6 months after receipt, 3 weeks after reconstitution.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Samples: serum and plasma samples should be non-hemolyzed and assayed immediately. If not assayed, samples can be stored at -80°C for up to 30 days.

Reagent preparation: bring all reagents to room temperature prior to assay. Reconstitute the AcAc Reagent tube with 1000 µL dH_2O (final 10 mM). Unused AcAc Reagent is stable for three weeks when stored frozen at -20°C. During experiment, keep the HBDH enzyme on ice or in refrigerator (2-8°C).

AcAc Assay

1. *Standards.* Prepare 8 mM standard by mixing 5 µL AcAc standard with 45 µL distilled H_2O. Transfer 5 µL H_2O, 5 µL 8 mM AcAc standard in separate wells of a clear, flat-bottom, 96-well plate.

   Samples. Transfer 5 µL sample into two wells, one *Sample* well and one sample *Blank* well.

2. *Reaction.* Prepare Working Reagent for H_2O, Standard and *Sample* wells, by mixing 195 µL AcAc Buffer, 8 µL reconstituted AcAc Reagent and 0.5 µL HBDH Enzyme for each well. The Blank Reagent is prepared by mixing, for each *blank* well, 195 µL AcAc Buffer and 8 µL reconstituted AcAc Reagent (i.e., *no enzyme*).

   Add 195 µL Working Reagent to the H_2O, Standard and *Sample* wells. Add 195 µL Blank Reagent to Sample *Blank* wells. Gently tap plate to mix.

3. Incubate 5 min at room temperature. Read OD_{340nm}. Calculate the acetoacetic acid (AcAc) concentration from the OD values of the H_2O, 8 mM Standard, Sample and Sample Blank wells,

$$[\text{AcAc}] = \frac{OD_{BLANK} - OD_{SAMPLE}}{OD_{H2O} - OD_{STANDARD}} \times 8 \ \text{(mM)}$$

BOH Assay

1. *Standards*. Prepare 8 mM standard by mixing 5 µL BOH standard with 45 µL distilled H_2O. Transfer 5 µL H_2O, 5 µL 8 mM BOH standard in separate wells of a clear, flat-bottom, 96-well plate.

   Samples. Transfer 5 µL sample into two wells, one *Sample* well and one sample *Blank* well.

2. *Reaction*. Prepare Working Reagent for H_2O, Standard and *Sample* wells, by mixing 195 µL BOH Buffer, 8 µL BOH Reagent and 0.5 µL HBDH Enzyme for each well. The Blank Reagent is prepared by mixing, for each *blank* well, 195 µL BOH Buffer and 8 µL BOH Reagent (i.e., *no enzyme*).

   Add 195 µL Working Reagent to the H_2O, Standard and *Sample* wells. Add 195 µL Blank Reagent to Sample *Blank* wells. Gently tap plate to mix.

3. Incubate 15 min at room temperature and read OD_{340nm}. Calculate the 3-hydroxybutyric acid (BOH) concentration from the OD values of the sample, sample blank, Standard and H_2O,

$$[\text{BOH}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{H2O}} \times 8 \ \text{(mM)}$$

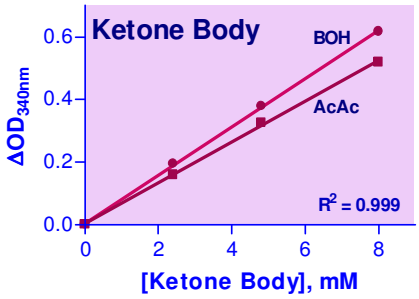
Total ketone body (TKB) concentration is calculated as,

$$[\text{TKB}] = [\text{AcAc}] + [\text{BOH}]$$

Note: if the calculated [AcAc] or [BOH] is higher than 8 mM, dilute sample in H_2O and repeat this assay. Multiply the results by the dilution factor.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting (multi-channel) devices. Clear flat-bottom 96-well plates (e.g. Corning Costar) and plate reader.



Standard Curves of Acetoacetic Acid (AcAc) and 3-Hydroxybutyric Acid (BOH)

LITERATURE

1. Nuwayhid, N.F., Johnson, G.F. and Feld, R.D. (1988). Kinetic measurement of the combined concentrations of acetoacetate and β-3-hydroxybutyrate in serum. Clin. Chem. 34/9, 1790-1793.
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3. Siegel, L., Robin, N.I. and McDonald, L.J. (1977). New approach to determination of total ketone bodies in serum. Clin. Chem. 23/1, 46-49.

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