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Product Information

Ketone Body Assay Kit

Catalog Number **MAK134**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Ketone bodies, 3-hydroxybutyric acid (BOH) and acetoacetic acid (AcAc), are produced in the liver primarily 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 hypoglycemia, growth hormone deficiency, alcohol or salicylate intoxication, and inborn errors of metabolism.

In this kit, AcAc and BOH levels are determined using an enzymatic assay based on 3-hydroxybutyrate dehydrogenase catalyzed reactions, in which the change in NADH absorbance measured at 340 nm is directly related to either the AcAc and BOH concentrations. This kit can be used various sample types, including serum, plasma, urine, and other biological samples.

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

Components

This kit is sufficient for up to 100 assays of 3-hydroxybutyric acid (BOH) and up to 100 assays of acetoacetic acid (AcAc) in 96 well plates.

AcAc Buffer Catalog Number MAK134A	20 mL
BOH Buffer Catalog Number MAK134B	20 mL
AcAc Reagent Catalog Number MAK134C	1 vl
BOH Reagent Catalog Number MAK134D	1 mL
AcAc Standard, 80 mM Catalog Number MAK134E	200 µL
BOH Standard, 80 mM Catalog Number MAK134F	200 µL
HBDH Enzyme Catalog Number MAK134G	120 µL

Reagents and Equipment Required but Not Provided.

- Clear 96 well flat-bottom plate suitable for use in UV absorbance assays (Catalog No. CLS3635 or equivalent).
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents and standards.

AcAc Reagent – Reconstitute with 1,000 µL of water to create a 10 mM solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within three weeks of reconstitution.

Storage/Stability

The kit is shipped on dry ice and storage at –20 °C, protected from light, is recommended.

Procedures

Serum and plasma samples should be non-hemolyzed and, for best results, assayed immediately. If not assayed, samples can be stored at –80 °C for up to 30 days.

Bring all reagents except for the HBDH enzyme to room temperature prior to assay. The HBDH should be kept on ice during use.

AcAc Assay

Standards for AcAc Detection

Add 5 μL of the 80 mM AcAc Standard to 45 μL of water to prepare an 8 mM AcAc Standard solution. Transfer 5 μL of the 8 mM standard and 5 μL of water into separate wells of 96 well plate.

Sample Preparation

Samples can be assayed directly. Aliquot 5 μL of sample into two separate wells of a 96 well plate. One well will be used for the sample activity and one for the sample blank.

AcAc Assay Reaction

1. Set up the AcAc Reaction Mixes according to the scheme in Table 1. 195 μL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
AcAc Reaction Mixes

Reagent	Sample and Standards	Sample blank
AcAc Assay Buffer	195 μL	195.5 μL
AcAc Reagent	8 μL	8 μL
HBDH Enzyme	0.5 μL	–

2. Add 195 μL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction at room temperature for 5 minutes. Protect the plate from light during the incubation.

3. Measure the absorbance of the samples and standards at 340 nm (A_{340}).

Results

Calculate the acetoacetic acid (AcAc) concentration from the A_{340} values of the water blank, 8 mM Standard, Sample, and Sample Blank. The concentration of AcAc (mM) in a sample is calculated as:

$$\text{AcAc (mM)} = \frac{A_{\text{sample blank}} - A_{\text{sample}}}{A_{\text{water}} - A_{\text{standard}}} \times 8$$

Note: If the calculated AcAc for the sample is higher than 8 mM, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

BOH Assay

Standards for BOH Detection

Add 5 μL of the 80 mM BOH Standard to 45 μL of water to prepare an 8 mM BOH Standard solution. Transfer 5 μL of the 8 mM BOH standard and 5 μL of water into separate wells of 96 well plate.

Sample Preparation

Samples can be assayed directly. Aliquot 5 μL of sample into two separate wells of a 96 well plate. One well will be used for the sample activity and one for the sample blank.

BOH Assay Reaction

1. Set up the Reaction Mix according to the scheme in Table 2. 195 μL of the appropriate Reaction Mix is required for each reaction (well).

Table 2.
BOH Reaction Mixes

Reagent	Sample and Standards	Sample blank
BOH Assay Buffer	195 μL	195.5 μL
BOH Reagent	8 μL	8 μL
HBDH Enzyme	0.5 μL	–

2. Add 195 μL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction at room temperature for 15 minutes. Protect the plate from light during the incubation.

3. Measure the absorbance of the samples and standards at 340 nm (A_{340}).

Results

Calculate the 3-hydroxybutyric acid (BOH) concentration from the A_{340} values of the water blank, 8 mM Standard, Sample, and Sample Blank. The concentration of BOH in a sample is calculated as:

$$\text{BOH (mM)} = \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{standard}} - A_{\text{water}}} \times 8$$

Note: If the calculated BOH for the sample is higher than 8 mM, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Total ketone body (TKB) concentration is calculated as:

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above the standard	Dilute samples so readings are in range

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