

Version 1b Last updated 10 March 2026

ab272541

Ketone Body Assay Kit

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Ketone Body Assay Kit datasheet:

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For quantitative determination of Ketone Bodies (acetoacetate and 3-hydroxybutyrate).

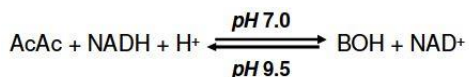
This product is for research use only and is not intended for diagnostic use.

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1. Overview

Ketone Body Assay Kit (ab272541) is a simple, direct and automation-ready procedure for measuring acetoacetic acid (AcAc) and 3-hydroxybutyric acid (BOH). This 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.

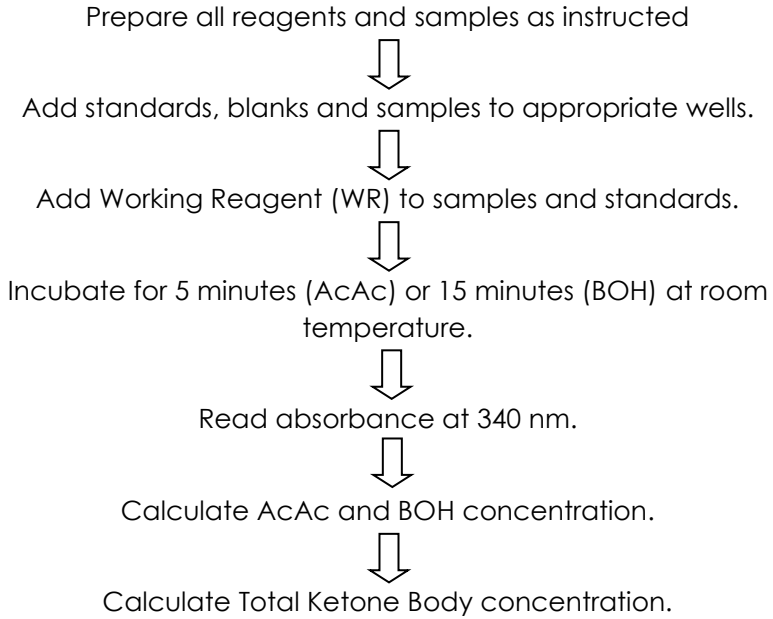


Sensitive and accurate: Uses 10 μL sample. Linear detection range of 0.12 to 8 mM for each ketone body in 96-well plate assay.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website www.abcam.com.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 6 months from receipt, providing components have not been reconstituted. 3 weeks after reconstitution.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
AcAc Buffer	20 mL	-20°C
AcAc Reagent	1 vial	-20°C
AcAc Standard	200 µL	-20°C
HBDH Enzyme	120 µL	-20°C
BOH Buffer	20 mL	-20°C
BOH Reagent	1 mL	-20°C
BOH Standard	200 µL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H₂O
- Multi-channel pipette
- 96-well clear plate with flat bottom
- Standard microplate reader - capable of reading absorbance at 340 nm

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 100 assays.

9.1 AcAc Reagent:

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 .

All other reagents are supplied ready to use.

Δ Note: During experiment, keep the HBDH enzyme on ice or in refrigerator ($2-8^\circ\text{C}$).

10. Standard Preparation

- Always prepare a fresh set of standards for every use.

10.1 For AcAc Assay:

Prepare 8 mM standard by mixing 5 μ L AcAc standard with 45 μ L distilled H₂O.

10.2 For BOH Assay:

Prepare 8 mM standard by mixing 5 μ L BOH standard with 45 μ L distilled H₂O.

11. Sample Preparation

Serum and plasma samples should be non-hemolyzed and assayed immediately.

Δ Note: If not assayed, samples can be stored at -80°C for up to 30 days.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

AcAc Assay:

12.1 Samples

12.1.1 Transfer 5 μL sample into appropriate wells (Sample Wells).

12.1.2 Transfer 5 μL sample into separate wells (Sample Blank Wells).

12.2 Standards

12.2.1 Transfer 5 μL 8 mM AcAc Standard into appropriate wells (Standard Wells).

12.2.2 Transfer 5 μL distilled H_2O into separate wells (H_2O Wells).

12.3 Working Reagent

– Immediately prior to starting the reaction as detailed in the table

12.3.1 Prepare enough Working Reagent (WR) for all dH_2O , standards and samples by mixing per reaction well: 195 μL AcAc Buffer, 8 μL reconstituted AcAc Reagent and 0.5 μL HBDH Enzyme.

12.3.2 Blank Reagent is prepared by mixing, for each blank well: 195 μL AcAc Buffer and 8 μL reconstituted AcAc Reagent.

Component	Working Reagent	Blank Reagent
AcAc Buffer	195 μL	195 μL
AcAc Reagent	8 μL	8 μL
HBDH Enzyme	0.5 μL	N/A

12.3.3 Add 195 μL Working Reagent to the dH_2O , Standard and Sample wells.

12.3.4 Add 195 μL Blank Reagent to Sample Blank wells.

12.3.5 Gently tap plate to mix.

12.3.6 Incubate 5 min at room temperature.

12.3.7 Read OD at 340 nm.

Δ Note: Use 96-well clear, flat-bottom plates.

BOH Assay:

12.4 Samples

12.4.1 Transfer 5 μL sample into well (Sample Wells).

12.4.2 Transfer 5 μL sample into well (Sample Blank Wells).

12.5 Standards

12.5.1 Transfer 5 μL 8 mM BOH Standard (Standard Wells).

12.5.2 Transfer 5 μL distilled H_2O (H_2O Wells).

12.6 Working Reagent

12.6.1 Prepare enough Working Reagent for all distilled H_2O , standards and samples by mixing per reaction well: 195 μL BOH Buffer, 8 μL BOH Reagent and 0.5 μL HBDH Enzyme.

12.6.2 Blank Reagent is prepared by mixing, for each blank well: 195 μL BOH Buffer and 8 μL BOH Reagent.

Component	Working Reagent	Blank Reagent
BOH Buffer	195 μL	195 μL
BOH Reagent	8 μL	8 μL
HBDH Enzyme	0.5 μL	N/A

12.6.3 Add 195 μL Working Reagent to the dH_2O , Standard and Sample wells.

12.6.4 Add 195 μL Blank Reagent to Sample Blank wells.

12.6.5 Gently tap plate to mix.

12.6.6 Incubate 15 min at room temperature.

12.6.7 Read OD at 340 nm.

Δ Note: Use 96-well clear, flat-bottom plates.

13. Calculations

AcAc Assay:

Calculate AcAc concentration from the OD values of the dH₂O, Standard, Sample and Sample Blank wells.

$$[\text{AcAc}] = ((\text{OD}_{\text{Blank}} - \text{OD}_{\text{Sample}}) / (\text{OD}_{\text{dH}_2\text{O}} - \text{OD}_{\text{Standard}})) \times 8 \text{ (mM)}$$

OD_{Sample} = OD value of the sample

OD_{Blank} = OD value of blank

OD_{Standard} = OD value of the standard

OD_{dH₂O} = OD value of water

BOH Assay:

Calculate BOH concentration from the OD values of the dH₂O, Standard, Sample and Sample Blank wells.

$$[\text{BOH}] = ((\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{dH}_2\text{O}})) \times 8 \text{ (mM)}$$

OD_{Sample} = OD value of the sample

OD_{Blank} = OD value of blank

OD_{Standard} = OD value of the standard

OD_{dH₂O} = OD value of water

Total ketone body (TKB) concentration is calculated as,

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

ΔNote: if the calculated [AcAc] or [BOH] is higher than 8 mM, dilute sample in H₂O and repeat this assay. Multiply the results by the dilution factor.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

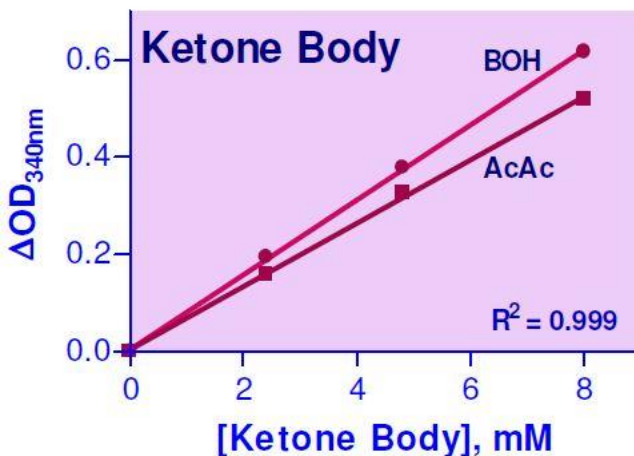


Figure 1. Example of Acetoacetic Acid (AcAc) and 3-Hydroxybutyric Acid (BOH) standard curve.

15. Notes

Technical Support

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