**Intended use**

Autokit Total Ketone Bodies test is an in vitro assay for the quantitative determination of total ketone bodies \(\text{acetacetate (AcAc)} + 3\text{-hydroxybutyrate (3-HB)}\) in serum or plasma.

**Summary and explanation of the test**

The ketone bodies assays should include, more accurately, acetone, AcAc, and 3-HB. However, it is a general practice in clinical lab to measure total ketone bodies as a sum of AcAc and 3-HB. Ketone bodies are substances metabolically produced from fatty acids in liver. The ketone bodies assays are used for diagnosis of diabetes since the concentration in blood increase in hyperlipolysis due to disorder in sugar metabolism. The ketone bodies assays are also used in the field of surgery such as liver transplantation since the ketone body ratio \(\text{AcAc}/3\text{-HB}\) in arterial blood reflects liver reserve capacity. Autokit Total Ketone Bodies is a reagent to measure total ketone bodies with high sensitivity and high specificity by utilizing cyclic enzymatic reactions. The concentration of AcAc can be calculated with a 3-HB value obtained by using Autokit 3-HB.

**Reagents**

**Autokit Total Ketone Bodies R1 Set**

- **R1a: Buffer**
  - 2 x 27 mL
  - Store at 2–10 °C (Do not freeze)
  - 20 mmol/L, Phosphate buffer, pH 7.0, containing 0.018 % sodium azide.

- **R1b: Thio-NAD**
  - 2 x for 27 mL
  - Store at 2–10 °C
  - 4.27 mmol/L, β-thionicotinamide adenine dinucleotide, oxidized form (Thio-NAD), when reconstituted.

**Autokit Total Ketone Bodies R2 Set**

- **R2a: Diluent**
  - 2 x 9 mL
  - Store at 2–10 °C
  - 0.2 mol/L Good’s buffer, pH 9.0, containing 0.053 % sodium azide.

- **R2b: Enzyme**
  - 2 x for 9 mL
  - Store at 2–10 °C
  - 3200 IU/mL 3-Hydroxybutyrate dehydrogenase (3-HBDH), from Alcaligenes and 2.65 mmol/L β-nicotinamide adenine dinucleotide disodium, reduced form (NADH), when reconstituted.

**Principle of the method**

When a sample is mixed with R1 and R2, AcAc and 3-HB in the sample are converted to 3-HB and AcAc, respectively, in the presence of 3-HBDH, NADH, containing Thio-NAD. 3-HB and AcAc produced in the enzymatic reactions are, then, converted to AcAc and 3-HB, respectively. During these cyclic reactions, NAD and Thio-NADH are produced. By measuring the rate of Thio-NADH production spectrophotometrically, the concentration of total ketone bodies in the sample is determined.

**Reagent preparation**

- **R1**: Dissolve one bottle of R1b with one bottle of R1a. The reconstituted solution is stable for 3 weeks at 2–10 °C.
- **R2**: Dissolve one bottle of R2b with one bottle of R2a. The reconstituted solution is stable for 3 weeks at 2–10 °C.

**Physical or chemical indications of instability**

The presence of precipitates in the reagents or values of control sera outside the manufacturer’s acceptable range may be an indication of reagent instability.

**Correlation**

- **Sample**
  - Serum
  - Plasma
- **Correlation coefficient**
  - Serum: \(r = 0.999 (n = 55)\)
  - Plasma: \(r = 0.999 (n = 52)\)
- **Regression equation**
  - \(y = 0.98x - 5.1\)
  - \(y = 1.02x - 6.4\)
- **Y**
  - \(y\) = Autokit Total Ketone Bodies
    - (Standard method, µmol/L)
  - \(x\) = A product from company A
    - (Enzymatic method, µmol/L)
Warnings and precautions

- For in vitro diagnostic use.
- Not to be used internally in humans and animals.
- Do not use reagents past the expiration date stated on each reagent container label.
- Do not use reagents that were frozen in error. Such reagents may give false results.
- Do not use reagents for any purpose other than those described herein.
- Do not use the containers and other materials in the kit for any purpose other than those described herein.
- Be careful not to cut yourself with the aluminium cap when removing it from the vial.
- Use Wako’s Ketone Body Calibrator for calibration. Read the instruction sheet in the package of the calibrator thoroughly before use.
- Buffer and Diluent contain sodium azide as a stabilizer. Sodium azide may react with lead or copper plumbing to form explosive compounds. Even though the reagents contain minute quantities of sodium azide, drains should be flushed well with large amount of water, when discarding the reagents.
- If the reagents come in contact with mouth, eye or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
- When discarding the reagents, dispose of them according to local or national regulations.

Quality Control

A quality control program is recommended for all clinical laboratories.

References

3. Fritzsche, I., Bührdel, P., Metzger, R., Böhme, H.-J. Stability of Ketone Bodies in Serum in Dependence on Storage Time and Storage Temperature, Clin. Lab. 47, 399-403 (2001)-

Ordering Information

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Product Description</th>
<th>Package</th>
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<tbody>
<tr>
<td>415-73301</td>
<td>Autokit Total Ketone Bodies R1 Set</td>
<td>R2a: 2 x 27 mL, R1b: 2 x 27 mL</td>
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<tr>
<td>413-73601</td>
<td>Autokit 3-HB R2 Set</td>
<td>R2a: 2 x 9 mL, R2b: 2 x 9 mL</td>
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<td>412-73791</td>
<td>Ketone Body Calibrator • 300 (3-HB: 300 µmol/l)</td>
<td>CAL: 4 x 5 mL</td>
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</table>

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