**Intended use**

Autokit Total Ketone Bodies test is an *in vitro* assay for the quantitative determination of total ketone bodies ([acetooacetate (AcAc) + 3-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 assay is also used in the field of surgery, e.g., liver transplantation since the ketone body ratio (AcAc/3-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 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 0.063% sodium azide

R2b: Enzyme 2 x for 9 mL
- Store at 2-10°C
3200 IU/mL 3-Hydroxybutyrate dehydrogenase (3-HBDH) 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, and 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-NAD are oxidized to Thionicotinamide adenine dinucleotide, oxidized form (Thio-NAD), when reconstituted.

**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.

**Instruments**

The reagent is designed to be used on commercially available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications. A validation by the user in practice at the customer’s site in the form of measurements of adequate control or patient sera in sufficient number is indispensable.

**Standard procedure**

**Temperature:** 37°C

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serum</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r = 0.999 ( n=55 )</td>
<td>r = 0.999 ( n=52 )</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y= 0.98x –5.1</td>
<td>y= 1.02x - 6.4</td>
</tr>
<tr>
<td>µmol/L</td>
<td>µmol/L</td>
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</tbody>
</table>

*Product from company A*

**Limitations of the procedure**

When total ketone bodies concentration in a sample exceeds the upper limit of linearity, dilute the sample with saline solution, repeat assay and multiply result by the dilution factor.

**Expected values**

28-120 µmol/L in serum or plasma.

**Precautions on procedure**

1) **Samples**

- Perform the total ketone bodies assay immediately after blood collection due to the instability of the AcAc in the sample. Store samples in a refrigerator or a freezer, if immediate assay cannot be done. Upon separation of blood cells immediately after blood collection, AcAc is stable for 2 hours at room temperature and for 3 days at –20°C.

- Hemolysis gives slightly falsely negative results.

- Ascorbic acid and bilirubin do not have a significant effect on the assay.

2) **Interfering substances**

- Heparin, citrate, oxalate, EDTA and sodium fluoride do not affect measurements when they are used in their respective usual quantities.

**Performance characteristics**

**Sensitivity**

- When purified water is used as a sample, the absorbance change (ΔE/min) is 0.03 or less.

- When a standard solution (200 µmol/L 3-HB) is used as a sample, the absorbance change (ΔE/min) is 0.02-0.40 against the blank.

**Specificity**

- When a sample of known concentration is assayed, the measured value is within ±10% of the known concentration.

**Precision**

- When a sample is assayed 5 times in a run, CV is within 5%.

**Measurable range**

- Total Ketone Bodies concentration
- Standard method: 3-1000 µmol/L
- High sensitivity method: 0.2-200 µmol/L

**Correlation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serum</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
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<td>A product from company A</td>
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</tbody>
</table>

**References**

- 1
- 2
- 3

**URL:** [www.wako-chemicals.de](http://www.wako-chemicals.de)
Warnings and precautions

- For in vitro diagnostic use.
- Not to be used internally in humans and animals.
- Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures or for other purposes.
- Store the reagents under the specified conditions. 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.
- After opening the reagents, it is recommended to use them immediately. When the opened reagents are stored, cap the bottles and keep them under the specified conditions.
- 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 stabiliser. 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


Ordering information

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Product</th>
<th>Package</th>
</tr>
</thead>
<tbody>
<tr>
<td>415-73301</td>
<td>Autokit Total Ketone Bodies R1 Set</td>
<td>R1a: 2 x 27 mL</td>
</tr>
<tr>
<td></td>
<td>(Autokit T-KB R1 Set)</td>
<td>R1b: 2 x for 27 mL</td>
</tr>
<tr>
<td>413-73601</td>
<td>Autokit 3-HB R2 Set</td>
<td>R2a: 2 x 9 mL</td>
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<tr>
<td></td>
<td>(3-HB: 300µmol/L)</td>
<td>R2b: 2 x for 9 mL</td>
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<tr>
<td>412-73791</td>
<td>Ketone Body Calibrator • 300</td>
<td>CAL: 4 x 5 mL</td>
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<tr>
<td></td>
<td>(3-HB: 300µmol/L)</td>
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