

415-73301
0524D4

Intended use

Autokit Total Ketone Bodies test is an *in vitro* assay for the quantitative determination of total ketone bodies [acetoacetate (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 hyperlipidosis 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 (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 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.

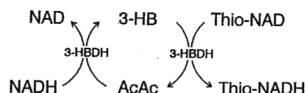
Autokit 3-HB R2 Set

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, 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-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

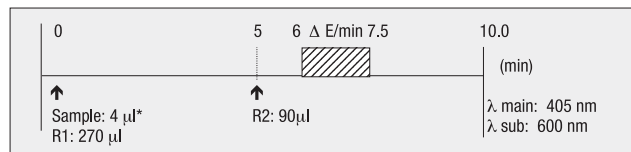
The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

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



*In the case of high sensitivity method, sample volume is 17 µL.

The above standard procedure is an example. Instrument applications are available upon request.

Results

The final results are automatically calculated and printed in concentration. The results are given in µmol/L.

Expected values

28–120 µmol/L in serum or plasma².

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.

Precautions on procedure

- 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 °C³.
 - Hemolysis gives slightly falsely negative results.
 - Ascorbic acid and bilirubin do not have a significant effect on the assay.
- Interfering substances Heparin, citrate, oxalate, EDTA and sodium fluoride do not affect measurements when they are used in their respective usual quantities.

Performance data

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

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

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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.

Labelling according to Regulation (EC) No. 1272/2008

- The product is classified and labelled according to the CLP regulation.
- The product does not contain a SVHC according to REACH, Article 57 >0,1 %

Hazard-determining components of labelling (R1b)

- Adenosine 5'-(trihydrogen diphosphate), 5'-5'-ester with 3-(aminothioxomethyl)-1-β-D-ribofuranosylpyridinium--ate

Hazard pictograms



Warning

GHS07

Hazard statements

- Harmful if swallowed, in contact with skin or if inhaled.
- Causes skin irritation.
- May cause respiratory irritation.

Precautionary statements

- Avoid breathing dust/fume/gas/mist/vapours/spray.
- Wash thoroughly after handling.
- Wear protective gloves / protective clothing.
- IF exposed or concerned: Call a POISON CENTER/doctor.

Hazard-determining components of labelling (R2a)

- Sodium hydroxide

Hazard pictograms



Warning

GHS07

Hazard statements

- Causes skin irritation.
- Causes serious eye irritation.

Precautionary statements

- Wash thoroughly after handling.
- Wear eye protection / face protection.
- IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- If eye irritation persists: Get medical advice/attention.

Hazard-determining components of labelling (R2b)

- 3-Hydroxybutyrate dehydrogenase

Hazard pictograms



Danger

GHS08

Hazard statements

- May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statements

- Avoid breathing dust/fume/gas/mist/vapours/spray.
- [In case of inadequate ventilation] wear respiratory protection.
- IF INHALED: Remove person to fresh air and keep comfortable for breathing.
- If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

Quality Control

A quality control program is recommended for all clinical laboratories.

References

1. Hirano, T. Modern Med. Lab., 19 (13), 1113-1117 (1991), in Japanese.
2. Hidaka, H. and Shigeta, Y., Jpn. J. Clin. Med., 53, supplementary issue, 603-605 (1995), in Japanese.
3. Fritzsche, I., Bährdel, P., Melcher, 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

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