Autokit 3-HB

Cyclic Enzymatic Method

For the quantitative determination of 3-hydroxybutyrate (3-HB) in serum or plasma

417-73501
1207 D2

Intended use

Autokit 3-HB test is an *in vitro* assay for the quantitative determination of 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 labs to measure AcAc, 3-HB and 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 increases 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 (AcAc/3-HB) in arterial blood reflects liver reserve capacity. Autokit 3-HB is a reagent to measure 3-HB with high sensitivity and high specificity by utilizing cyclic enzymatic reactions.

Reagents

Autokit 3-HB R1 Set

R1a:Buffer	2 x 27 mL	Store at 2-10°C (Do not freeze)
	20 mmol/L Phosphate buffer, pH7.0 , containing 5 IU/mL acetoacetate decarboxylase (AADC) from *Bacillus* and 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

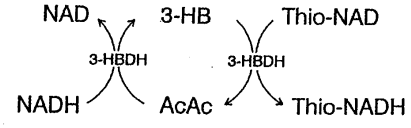
R2a: Diluent	2 x 9 mL	Store at 2-10°C
	0.2 mol/L Good's buffer, pH 9.0, containing 0.053% sodium azide.	
R2b: Enzyme	2 x for 9 mL	Store at 2-10°C
	3200 IU/mL 3-Hydroxybutyrate dehydrogenase (3-HBDH), from *Alcaligenes* and 2.65 mmol/L β-nicotinamide adenine dinucleotide disodium, reduced form (NADH), when reconstituted.	

Principle of the method

When a sample is mixed with R1, AcAc in the sample is broken down to acetone by AADC.

$$AcAc \xrightarrow{AADC} Acetone + CO_2$$

Upon addition of R2, 3-HB in the sample is oxidized in the presence of 3-HBDH and Thio-NAD. This oxidation triggers the cyclic reactions. Since the original AcAc in the sample has been removed, only 3-HB is assayed by measuring the rate of Thio-NADH production spectrophotometrically.



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

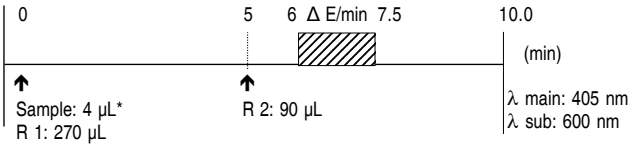
The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent instability.

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

0-74 µmol/L in serum or plasma[2].

Limitations of the procedure

When 3-HB 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

1) Samples
(a) Perform the 3-HB assay immediately after blood collection. Store samples in a refrigerator or a freezer if the immediate assay cannot be done.
(b) Hemolysis gives slightly falsely negative results.
(c) 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 effect measurements when they are used in their respective usual quantities.

Performance data

Sensitivity

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

3-HB 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= 1.02x – 3.2	y=0.99x – 6.3
y	Autokit 3-HB (Standard method, µmol/L)	
x	A product from company A (Enzymatic method, µmol/L)	

Manufactured by:

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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 aluminum 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

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., Bührdel, 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
417-73501	Autokit 3-HB 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: 300µmol/L)	CAL: 4 x 5 mL

Manufactured by:

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