FUJIFILM

Code No. 415-73301
411-73401

Autokit Total Ketone Bodies

(Cyclic Enzymatic Method)

For Research Use Only. Not for use in diagnostic procedures.

Intended use

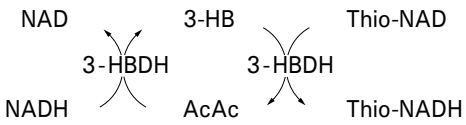
The Autokit Total Ketone Bodies is an *in vitro* assay for the quantitative determination of total ketone bodies [acetoacetone (AcAc) + 3-hydroxybutyrate (3-HB)] in serum or plasma.

Summary and explanation of the test

The Autokit Total Ketone Bodies is a reagent to measure total ketone bodies with high sensitivity and high specificity by utilizing cyclic enzymatic reactions.

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.



Reagents

(1) Thio–NAD 2 × for 27 mL
When reconstituted
4.27 mmol/L β-Thionicotinamide adenine dinucleotide, oxidized form (Thio-NAD)
Store at 2-10℃.

(2) Buffer 2 × 27 mL
20 mmol/L Phosphate buffer, pH 7.0, containing 0.018% sodium azide.
Store at 2-10℃.

(3) Enzyme 2 × for 9 mL
When reconstituted
3200 IU/mL 3-Hydroxybutyrate dehydrogenase (3-HBDH) from *Alcaligenes*
2.65 mmol/L β-nicotinamide adenine dinucleotide disodium, reduced form (NADH)
Store at 2-10℃.

(4) Diluent 2 × 9 mL
0.2 mol/L Good's buffer, pH 9.0, containing 0.053% sodium azide.
Store at 2-10℃.

Warnings and precautions

(1) For Research Use Only. Not for use in diagnostic procedures.
(2) 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.
(3) Operate the instruments according to operator's manuals under appropriate conditions. Consult the instrument manufacturer for details.
(4) Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on each reagent container label.
(5) Do not use reagents which were frozen in error. Such reagents may give false results.
(6) 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.
(7) Do not use the containers and other materials in the package for any purpose other than those described herein.
(8) Use Wako's Ketone Body Calibrator for preparation of a calibration curve. Read the instruction sheet in the package of the calibrator thoroughly before use.
(9) When discarding the reagents, dispose of them according to local or national regulations.
(10) The Buffer and Diluent contain 0.018%, 0.053% sodium azide respectively, as a preservative. Sodium azide may react with copper or lead plumbing to form explosive compounds. Even though the reagents contain minute quantity of sodium azide, drains should be flushed well with a large amount of water, when discarding the reagents.
(11) If the reagents come in contact with the mouth, eyes or skin, wash off immediately with a large amount of water. Consult a physician if necessary.
(12) Be careful not to cut yourself with the aluminum cap when removing it from the vial.

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 such as Wako 30R analyzer. Refer to the operating manual for a description of instrument operation, specifications and calibration.

Specimen collection and preparation

(1) Samples
(a) 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℃.[2]
(b) Hemolysis gives slightly falsely negative results.
(c) Ascorbic acid and bilirubin do not have a significant effect on the assay.

(2) Interfering substances
(a) Heparin, citrate, oxalate, EDTA, and sodium fluoride do not affect measurements when they are used in their respective usual quantities.

Warning/Biohazard

It is recommended that specimen collection be carried out in accordance with NCCLS Document M29-A3. No known test method can offer complete assurance that human blood samples will not transmit infection. Therfore, all blood derivatives should be considered potentially infectious.

Procedure for Wako 30R analyzer

Materials supplied

Refer to the section entitled ''Reagents.''

Materials required but not supplied

Wako 30R analyzer
Ketone Body Calibrator
Catalog No. 412-73791 300 μmol/L
Catalog No. 418-73891 40 μmol/L
All analyzer applications should be validated in accordance with CLIA recommendations. For further assistance call Wako Diagnostics Technical Service Department at 877-714-1924.

Reagent preparation

Reagent 1 : Dissolve one bottle of Thio-NAD with one bottle of Buffer. The reconstituted solution is stable for 3 weeks at 2-10℃.
Reagent 2 : Dissolve one bottle of Enzyme with one bottle of Diluent. The reconstituted solution is stable for 3 weeks at 2-10℃.

Test procedure
Parameter setting (Wako 30R)

Temperature : 37℃

TBA-30R / WAKO-30R	
Reagent	Total Ketone Bodies
TEST	Total Ketone Bodies
PAGE-2	
REACTION MODE	RATE
TEST WAVELENGTH	404
BLANK WAVELENGTH	604
TEST READ TIMING	38 - 44
BLANK READ TIMING	0 - 0
SAMPLE VOLUME	4.0 μL*3
REAGENT D	0
REAGENT 1	270 μL - Pos. - 50 mL
REAGENT 2	90 μL - Pos. - 50 mL
STIRRER	ON
PAGE-3	
CALIBRATION MODE	LINEAR
FACTOR	0
SLOPE FACTOR	100%
BASE FACTOR	0
BLANK #	3
STANDARD #	3
	POS-CONC-ABS
BLANK	0 - 0 -
STANDARD 1	*1 - *2 (300) -
PAGE-4	
REACTION MODE	UP
CALIBRATOR'S ABSORBANCE WINDOW	
SAMPLE (LOW / HIGH)	0 / 1300
BLANK (LOW / HIGH)	0 / 0
RATIOS	
REACTION MODE	RATE

*1 : Input the position of calibrator.
*2 : Input the conc. of the calibrator.
*3 : Sample volume : for the high sensitive procedure, use 17μL of sample, instead of 4μL

Results

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

Quality control

A quality control program is recommended for all laboratories. The analysis of control material in both low and high ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

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 the result by dilution factor.

Performance characteristics

Accuracy

The accuracy of the assay was demonstrated by a control recovery study.

	Expected Conc. (μmol/L)	Observed Conc. (μmol/L)	Recovery (%)
Sample 1	40	40	100%
Sample 2	150	148	99%
Sample 3	300	296	99%

Precision

Within run precision (standard procedure)

Sample #	Replicates	Mean (μmol/L)	s.d.	CV %
1	21	74.5	1.21	1.62
2	21	148.8	2.00	1.34
3	21	300.4	5.77	1.92

Within run precision (high sensitive procedure)

Sample #	Replicates	Mean (μmol/L)	s.d.	CV %
1	21	40.1	0.7684	1.9
2	21	70.5	0.9284	1.3

Total precision (standard procedure)

# days	Mean (μmol/L)	s.d.	CV %	S_{WT}	S_T
21	76.18	1.9156	2.51	0.94	1.92
21	157.85	4.2257	2.72	2.10	4.22
21	303.52	3.9615	1.31	2.36	3.96

Total precision (high sensitive procedure)

# days	Mean (μmol/L)	s.d.	CV %	S_{WT}	S_T
21	40.26	0.99	2.47	0.55	1.00
21	73.24	1.94	2.65	0.71	1.94

Sensitivity

The lower limit of measurement is 4.13 μmol/L for the 4 μL sample volume and 1.38 μmol/L for the high sensitive (17 μL) sample volume.

Measurable range

Standard method : 3-1000 μmol/L
High sensitivity method : 0.2-200 μmol/L

Linearity

The linearity of the Autokit Total Ketone Bodies is 1.2 μmol/L (the lower limit) to 1,185 μmol/L in the case of the 4 μL sample volume and from 1.38 μmol/L (the lower limit) to 275 μmol/L in the case of the 17 μL sample volume. If the total ketone bodies concentration exceeds the upper limit of the measurable range, dilute the sample with saline, repeat the assay and multiply the result by the dilution factor.

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)	Autokit Total Ketone Bodies (Standard method, μmol/L)
x	A product from Company A (Enzymatic method, μmol/L)	A product from Company A (Enzymatic method, μmol/L)

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)

Ordering information

Code No.	Product	Package
415-73301	Autokit Total Ketone Bodies R1 Set Thio–NAD 2 × for 27 mL Buffer 2 × 27 mL	2 × for 27 mL
411-73401	Autokit Total Ketone Bodies R2 Set Enzyme 2 × for 9 mL Diluent 2 × 9 mL	2 × for 9 mL
412-73791	Ketone Body Calibrator 300 (3–Hydroxybutyrate: 300 μ mol/L)	4 × 5 mL
418-73891	Ketone Body Calibrator 40 (3–Hydroxybutyrate: 40 μ mol/L) [for high sensitivity method]	4 × 5 mL

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415-73301F

Manufactured by
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