FUJIFILM

# Autokit 3-HB

(Cyclic Enzymatic Method)

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

Code No. 417-73501
413-73601

## Intended use

The Autokit 3-HB is an *in vitro* assay for the quantitative determination of 3-hydroxybutyrate (3-HB) in serum or plasma.

## Summary and explanation of the test

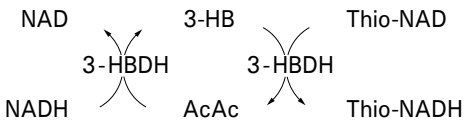
The Autokit 3-HB is reagent to measure 3-HB with high sensitivity and high specificity by utilizing cyclic enzymatic reactions.

## Principle of the method

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

$$\text{AcAc} \xrightarrow{\text{AADC}} \text{Acetone}$$

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.




## 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 5 IU/mL acetoacetate decarboxylase (AADC) from *Bacillus*
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 Hitachi 917s analyzer.
Refer to the operating manual for a description of instrument operation, specifications and calibration.

## Specimen collection and preparation

(1) Samples
(a) Perform the 3–HB assay immediately after blood collection. Store samples in a refrigerator or a freezer if 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
(a) Heparin, citrate, oxalate, EDTA, and sodium fluoride do not affect measurements when they are used in their respective usual quantities.

## Procedure for Hitachi 917s analyzer

### Materials supplied

Refer to the section entitled ''Reagents.''

### Materials required but not supplied

Hitachi 917s analyzer
Quality control material
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 1-877-714-1924.

### 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** (Hitachi917s)

Temperature : 37℃

| Reagent | | Autokit 3-HB |
|---|---|---|
| ANALYZE | | |
| CH TEST/TYPE | | 3-HB |
| ASSAY | | RateA - 10 |
| POINT | | 20 - 25 - 0 - 0 |
| WAVELENGTH | (SUB/MAIN) | 600 / 405 |
| SAMPLE VOL. | (NORMAL) | 2.3*4- 0.0 - 0 |
| | (DEC.) | |
| | (INC.) | |
| DILUENT | | ($H_2O$) - 0 |
| REAGENT VOL. | R1 | 150 - 0 - ( ) - 0 |
| | R2 | 0 - 0 - ( ) - 0 |
| | R3 | 50 - 0 - ( ) - 0 |
| | R4 | 0 - 0 - ( ) - 0 |
| ABS LIMIT | | 13000 Increase |
| PROZONE LIMIT | | |
| CELL DET. | | DET.1 |
| CALIBRATION | | |
| CALIB TYPE | | LINEAR |
| POINT | | 2 / 2 |
| WEIGHT | | 0 |
| AUTO CALIBRATION | | 0 |
| SD LIMIT | | 999.9 |
| DUPLICATE LIMIT | | 500 |
| SENSITIVITY LIMIT | | 0 |
| S1 ABS LIMIT | | −32000 / 32000 |
| RANGE | | |
| TEST # | | |
| UNIT | | μmol/L |
| REPORT NAME | | |
| DATA MODE | | On Board |
| CONTROL INTERVAL | | |
| INST. FACTOR | | a = 1.0 b = 0.0 |
| TECHNICAL LIMIT | | |
| EXPECTED VALUES | | |

| STD CONC. | CONC. | POS. | VOL. | PREDIL |
|---|---|---|---|---|
| 1 | 0.0 | $H_2O$ | 2.3*4 | 0.0 |
| 2 | *1*3 | *2 | 2.3*4 | 0.0 |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |

*1 : Input the assigned value of the calibrator 300.
*2 : Input the position of the calibrator.
*3 : In the case of high sensitivity method, input the assigned value of the calibrator 40.
*4 : For the high sensitivity method, the specimen volume is 10μL, instead of 2.3μL.

**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 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.

## Performance characteristics

(1) Accuracy
When a sample of known concentration is assayed, the measured value is within ±10% of the known concentration.
(2) Precision
When a sample is assayed 5 times in a run, CV is within 5%.
(3) 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.
(4) 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) | Autokit 3–HB (Standard method, μmol/L) |
| x | A product from Company A (Enzymatic method, μmol/L) | A product from Company A (Enzymatic method, μmol/L) |

## Reference

(1) Hirano, T, Modern Med. Lab., **19** (13), 1113–1117 (1991). (in Japanese)

## Ordering information

| Code No. | Product | Package |
|---|---|---|
| 417-73501 | Autokit 3–HB R1 Set<br>Thio–NAD 2 × for 27 mL<br>Buffer 2 × 27 mL | 2 × for 27 mL |
| 413-73601 | Autokit 3–HB R2 Set<br>Enzyme 2 × for 9 mL<br>Diluent 2 × 9 mL | 2 × for 9 mL |
| 412-73791 | Ketone Body Calibrator 300<br>(3–Hydroxybutyrate: 300μmol/L) | 4 × 5 mL |
| 418-73891 | Ketone Body Calibrator 40<br>(3–Hydroxybutyrate: 40μmol/L)<br>[for high sensitivity method] | 4 × 5 mL |

Revision date ; Apr. 1, 2018

Manufactured by
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18.03.16K03