BIOHIT HealthCare

Innovating for Health

BIOHIT Active B12

(HoloTC)

INSTRUCTION FOR USE

REF 602290 IVD CE

For *in vitro* diagnostic use

Store at 2-8 °C Upon Receipt

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INSTRUCTIONS FOR USE **English**

BIOHIT Active B12 (HoloTC) REF 602290

1. INTENDED USE

BIOHIT Active B12 (HoloTC) test is a quantitative in vitro enzyme-immunoassay (EIA) for determination of holotranscobalamin (HoloTC) in human serum aiding in the diagnosis and treatment of vitamin B12 deficiency. The test can be conducted either manually or automatically and is intended to be used by healthcare professionals only.

2. INTRODUCTION

Three binding proteins are involved in the transport of vitamin B12 around the body - Intrinsic Factor (IF), transcobalamin (TC) and haptocorrin (HC). These binding proteins ensure the efficient uptake of the very small amounts of vitamin B12 available from the diet. When TC and HC bind vitamin B12 the resulting complexes are known as holotranscobalamin (HoloTC) and holohaptocorrin (HoloHC) to distinguish them from the proteins carrying no vitamin.

The major fraction in the circulation, HoloHC, represents 70-90% of vitamin B12 in the blood but is biologically inert. HoloTC represents only 10-30% of vitamin B12 circulating in the blood but is the only form of vitamin B12 that can be taken up by cells in the body. The TC protein alone transports vitamin B12 from its site of absorption in the ileum to tissues and cells. The vitamin is then internalised as the HoloTC (vitamin B12 bound to transcobalamin) complex via a specific receptor-mediated uptake. This process delivers vitamin B12 into the cells of the body and provides the vitamin as a co-enzyme for essential cellular functions such as DNA synthesis.
As HoloTC has a shorter circulating half-life compared to HoloHC the earliest change that occurs on entering negative vitamin B12 balance is very likely to be a decrease in serum HoloTC concentration[1].

The measurement of Total Serum B12 suffers from some limitations; in particular, most of the measured cobalamin is bound to biologically inert HC. Several studies have been published which conclude that HoloTC would be a better indicator of vitamin B12 status than Total Serum B12[2,3]. As expected, HoloTC levels are low in patients with biochemical signs of vitamin B12 deficiency[4].

Low values have been reported in vegetarians[5], vegans[6], and in populations with a low intake of vitamin B12[7]. Notably, low levels of HoloTC but not Total B12 in serum were reported in patients with Alzheimer's disease compared to levels in a healthy control group[8]. HoloTC levels reflect vitamin B12 status, independent of recent absorption of the vitamin[9].

3. PRINCIPLE OF THE TEST

The microtiter wells are coated with a highly specific monoclonal antibody of active B12 (holotranscobalamin). During the first incubation holotranscobalamin in serum specifically binds to the antibody-coated surface. In the second incubation the conjugate binds to any captured holotranscobalamin. The wells are then washed to remove unbound components. Bound holotranscobalamin is detected by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a colored end-product. The concentration of holotranscobalamin in pmol/L is directly related to the color generated and can be estimated by interpolation from a dose-response curve based on Calibrators.

4. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only. Safety Precautions

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions.
2. Do not pipette by mouth. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
3. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
4. The Calibrators, Controls, Conjugate, Pre-Treatment and Wash Buffer Concentrate contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides and is very toxic to aquatic life with long lasting effects. Dispose of solutions and materials according to local waste management legislation.
5. The Stop Solution contains sodium hydroxide. Avoid contact with skin, eyes and mucous membranes. Spillage should be mopped up with copious amounts of water. If contact with skin or eyes occurs, irrigate with water and seek medical attention immediately.
6. Material safety data sheets for all components contained in this kit are available on request from Biohit Oyj.
7. This product requires the handling of specimens and materials of human and animal origin. It is recommended that all human and animal sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Blood borne Pathogens Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

 CORROSIVE	Stop Solution	H314 P264 P280 P305+351 +338	Causes severe skin burns and eye damage. Wash hands thoroughly after handling. Wear protective gloves/protective clothing/eye protection/face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
 HARMFUL	Substrate	H302+312 +332 P260 P271 P280 P301+310 P304+340 P312	Harmful if swallowed, in contact with skin or if inhaled. Do not breathe dust/fume/gas/mist/vapours/spray. Do not eat, drink or smoke when using this product. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: Immediately call a POISON CENTER/doctor. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell.
	Wash Buffer Concen-trate (8X)	H302 H412 EUH032 P264 P270 P273 P301+310 P330	Harmful if swallowed. Harmful to aquatic life with long lasting effects. Contact with acids liberates very toxic gas. Wash hands thoroughly after handling. Do not eat, drink or smoke when using this product. Avoid release to the environment. IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

5. KIT CONTENTS

5.1 Conjugate solution CONJ

One 15 mL vial of alkaline phosphatase-labelled murine monoclonal antibody to human transcobalamin in Tris buffer with protein stabiliser. Preservative: < 0.1% (w/v) sodium azide. Ready-to-use.

5.2 Substrate Solution SUBS 

One 15 mL vial of para-NitroPhenyl Phosphate (pNPP), buffer solution. Ready-to-use. Do not expose to light during storage. N.B. HARMFUL.

5.3 Stop Solution STOP 

One 15 mL vial of 1M Sodium hydroxide, (pH >10).
Ready-to-use. N.B. CORROSIVE.

5.4 Washing Buffer Concentrate WASH 8x 

Two 25 mL vials of phosphate buffer. Preservative: 0.72% (w/v) sodium azide.
Dilute before use. N.B. HARMFUL.

5.5 Microplate

12 × 8 well microtiter (break apart) strips coated with anti-holotranscobalamin murine monoclonal antibody, in a resealable foil pack with desiccant. Do not combine strips/wells from different microplates, even if they would have the same lot number.

5.6 Blank Solution BLANK

One 1 mL vial of phosphate buffer with protein (bovine) stabilizer.

5.7 Calibrators CAL 1-5

Five 1 mL vials of phosphate buffer with protein (bovine) stabilizer containing HoloTC.
Preservative: < 0.1% (w/v) sodium azide. Ready-to-use. Do not expose to light during storage.
SEE VIAL LABELS FOR CONCENTRATIONS.

5.8 Controls CONTROL LOW CONTROL HIGH

One vial of control low and one vial of control high : 1 mL of phosphate buffer with protein (bovine) stabiliser containing HoloTC. Preservative: < 0.1% (w/v) sodium azide.
Ready-to-use. Do not expose to light during storage.

5.9 Pre-treatment Solution PRE

One 25 mL vial of citrate buffer. Preservative: < 0.1% (w/v) sodium azide. Ready-to-use.

5.10 Instruction for use

6. MATERIALS/EQUIPMENT REQUIRED BUT NOT PROVIDED

1. 96 well plate/strip reader with 405 nm filter.
2. Precision pipette(s) to dispense 100 µL. An 8-channel dispenser, or similar, to dispense approximately 250-300 µL for manual washing.
3. Glass/plastic measuring cylinder 1×200 mL.
4. Distilled/deionised water.
5. Paper towels.
6. Timer for 30, 35 and 60 minute intervals.

7. STANDARDISATION

There is currently no internationally recognized reference method or reference material for standardization. The BIOHIT Active B12 (HoloTC) Calibrators are traceable to internal reference standards which underwent a one-time value assignment.

8. STORAGE AND STABILITY

8.1 Opened (In-Use) Kit Stability

A kit was opened and reused on three occasions over a three month period with no adverse effect on kit performance. Following use, components must be returned to storage at 2-8°C.

8.2 Unopened kit stability

At 2-8°C unopened components are stable until as directed on the labels.

8.3 Handling and Procedural Notes

1. Store kit components at 2-8°C and use until the expiry date on the labels. Do not use expired reagents.
2. Each lot of reagents and calibrators has been standardised to produce the correct reaction. Do not interchange the reagents or calibrators between lots.
3. Calibrator concentrations are displayed on vial labels and may vary between lots.
4. Do not freeze kits.
5. Wash Buffer Concentrate must be diluted before use. All other reagents are ready-to-use.
6. Diluted Wash Buffer is stable for at least 3 months if microbial contamination is avoided. Return to 2-8°C storage after each use.
7. Replace surplus (unused) microtiter strips in the foil pack with the desiccant. Ensure seal is integral and return to 2-8°C, until required.
8. Do not expose the Calibrators, Controls or Substrate to light during storage.
9. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.

8.4 Indications of Deterioration

The Substrate should be colorless to pale yellow in color. Darker yellow coloring indicates contamination, and the reagent must be discarded. Turbidity or precipitation in any component indicates deterioration and the component should be discarded.

9. SPECIMEN COLLECTION AND STORAGE

1. The assay is recommended for human serum (including serum separator tubes).
2. Do not use grossly hemolysed or turbid samples.
3. Thoroughly mix thawed samples before assay and avoid repeated freeze/thawing.
4. Samples may undergo 3 freeze thaw cycles. Thawed samples should be centrifuged at ≥10,000g for 5 minutes before assaying.
5. Do not subject on-the clot or off-the clot samples to temperature above room temperature for longer than overnight (≤ 16 hours).
6. Samples may be stored at 2-8°C on-the clot for up to 3 days or off-the-clot for four weeks; for longer storage samples must be stored off-the-clot at -20°C for up to 6 months.
7. Prepare each sample prior to assay by adding an equal volume of Pre-Treatment to Sample e.g.150µL sample plus 150µL Pre-Treatment. Pre-treated samples may be stored capped for up to 24 hours at 2-8°C prior to assay.

10. TEST PROCEDURE

10.1 Preparation

Preparation for the Assay

Allow all kit components, including the microtiter strips, to warm up to 18-25°C for 30-60 minutes before use. Mix reagents by gentle inversion.

When stored at 2-8°C the wash buffer will precipitate (crystals may be visible). Before diluting in water, allow the wash buffer to warm up (can be placed in an incubator at 37°C if required to speed the process) until **NO** precipitation is evident to the naked eye.

Dilute the following reagent and mix thoroughly:

Reagent	**Volume**	**Add**
Wash Buffer Concentrate x 8	1 vial	175 mL distilled/deionised water

Calculate the number of microtiter strips required for the current assay and retain these in the microtiter strip holder. Return surplus strips to the resealable foil pack with the desiccant and store at 2-8°C until required. Ensure that all strips are securely held within the microtiter strip holder. Users may wish to number each strip along the top edge to aid identification. Retain the microtiter strip holder for future use.

Prepare each sample prior to assay by adding an equal volume of Pre-Treatment to sample e.g.150 µL sample plus 150µL Pre-Treatment. Pre-treated samples may be stored capped for up to 24 hours at 2-8°C prior to assay.

10.2 Assay protocol

1. Reference wells for identification.
2. Pipette 100 µL Calibrators in duplicate, Kit Controls in duplicate and pre-treated (50:50) patient samples in duplicate, into appropriate wells. Remember to change pipette tips between additions. This step should not exceed 15 minutes.
3. Incubate 60±10 minutes at 18-25°C.
4. Decant strip contents by quick inversion over a sink suitable for the disposal of biological materials, bearing in mind the potential infective hazard of the samples. Blot inverted strips well with paper towels. **Do not wash.**
5. Add 100 µL Conjugate to each well.
6. Incubate 35±5 minutes at 18-25°C.
7. Decant strip contents by quick inversion over a sink suitable for the disposal of biological materials. Blot inverted strips well with paper towels.
8. Wash wells **five times** with a minimum of 250 µL diluted Wash Buffer. **Decant and blot after each wash addition.**
9. Add 100 µL Substrate to each well.
10. Incubate 30±5 minutes at 18-25°C. **Do not decant.**
11. Add 100 µL Stop Solution to each well, in the same order and rate as the Substrate. Tap wells gently to mix.
12. Read strips at 405nm. Read within 120 minutes of addition of Stop Solution.

11. RESULTS

11.1. Calculation and interpretation

Plot the mean absorbance value of each Calibrator on the y-axis against the corresponding concentration in pmol/L on the x-axis. **CALIBRATOR CONCENTRATIONS ARE DISPLAYED ON VIAL LABELS. CONCENTRATION VALUES ARE ASSIGNED TO EACH LOT OF CALIBRATORS AND MAY VARY BETWEEN LOTS.**

The concentration (pmol/L) of each sample can be calculated by locating the point on the curve corresponding to the mean sample absorbance value and reading the corresponding concentration in pmol/L from the x-axis. This procedure can be performed manually using graph paper or using a plate reader with software incorporating curve fitting procedures. If using a plate reader with internal software, **a linear regression curve-fit algorithm** should be used.

A typical calibration plot (Figure 1.) is shown below for reference purposes, it must not be used for interpreting results. Samples with concentrations above 128 pmol/L are outside the range of the assay and should be recorded as >128 pmol/L and results must not be extrapolated. Individual sample replicates deviating less than 20% can be taken to indicate acceptability of the assay.

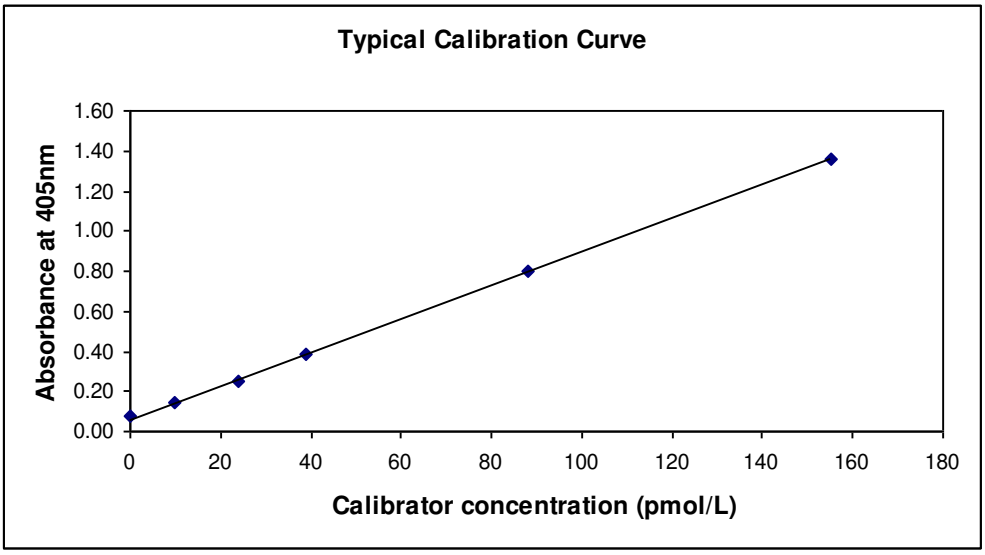

Figure 1. Typical Calibration Curve of BIOHIT Active B12 Assay.

Unit of Measure

The unit of measurement for the BIOHIT Active B12 (Holotranscobalamin) assay is pmol/L.

Measuring Interval (Reportable Range)

The measureable range of the assay is 10 pmol/L to 128 pmol/L.

11.2 Quality control

Ensure that adequate maintenance and calibration of the plate-reader is performed according to the manufacturer's instructions, and that the correct wavelength (405 nm) and curve-fit algorithm (linear regression) are employed.

Users should ensure they are fully acquainted with the instructions for the assay, particularly the Warnings and Precautions section, and the Handling and Procedural Notes. Users should demonstrate that they can obtain performance specifications for precision and reportable range of test results comparable to those established by the manufacturer before reporting patient test results. Ready-to-use Low and High Kit Controls must be run in duplicate in all assays to monitor the quality of the test procedure.

Assuming the precision specifications described by the manufacturer are met, failure of any Control to meet the Control specifications below renders the assay invalid and patient results should not be reported. The operator may repeat the assay, having reviewed their procedure, or contact the manufacturer. If repeating the assay, prepare a fresh dilution of each sample. Laboratories may wish to include in-house controls in each assay run. Store such control material at or below -20°C and avoid repeat freeze/thaw cycles. Preservatives such as sodium azide at <0.1% (w/v) will not affect sample results.

Reference ranges and appropriate cut-off points should be calculated for the specific populations served by users.

Table 1. Specifications for Low and High Controls.

Control Specifications	Control low	Control high
Mean of duplicate	15 to 35 pmol/L	36 to 84 pmol/L

11.3 Expected values

135 serum samples from asymptomatic apparently healthy donors with an age range of 18-75 years, comprising approximately equal numbers of males [n = 65] and females [n = 70], were tested in the BIOHIT Active B12 (Holotranscobalamin) EIA.

The overall mean BIOHIT Active B12 (Holotranscobalamin) concentration for this population was 72 pmol/L (range 15 to 147 pmol/L). On the basis of this reference population data, the reference range (central 95% of the results) is:

Reference Range	21 – 123 pmol/L

This reference range is suggested as a guideline only and each laboratory should establish its own reference range which may be unique to the population it serves depending upon geographical, patient, dietary or environmental factors or clinical practice.

12. PERFORMANCE DATA

Representative data; results in individual laboratories may vary.

12.1 Dilution Linearity

Based guidance from CLSI document EP6-A[10], the BIOHIT Active B12 (HoloTC) demonstrated linearity across the measuring range of the assay as demonstrated in a study from 5.3 to 156.0 pmol/L (rounded to 1 decimal place); results in individual laboratories may vary from these data).

12.2 Accuracy

A correlation study was performed with serum specimens from apparently healthy adults. All specimens were analysed using the BIOHIT Active B12 (HoloTC) EIA and another commercially available Holotranscobalamin assay according to the CLSI document EP9-A2[11]. Specimen concentrations ranged from 13.8 to 112.8 pmol/L in the assay. Table 2 shows the statistical values obtained.

Table 2. BIOHIT Active B12 EIA versus a commercially available assay.

BIOHIT Active B12 (HoloTC) EIA versus a commercially available assay	
Number of specimens	111
Slope of regression line (Passing-Bablok regression) (95% CI)	0.95 (0.89 to 1.01)
Y-intercept (Passing-Bablok regression) (95% CI)	8.39 (5.73 to 11.77)
Correlation coefficient (r) (Pearson) (95% CI)	0.93 (0.90 to 0.95)

12.3 Precision

Seven (7) human serum samples were assayed using 3 lots of reagents. Samples were assayed by 2 operators in replicates of 8, once a day for 5 days (total n=80). Data from this study are summarised in the Table 3.

Table 3. Precision data.

Sample	n	Lot	Operator	Mean (pmol/L)	Intra-assay %CV	Total %CV
1 A	80	1	1	17.8	7.5%	8.2%
			2	17.5	3.1%	9.3%
		2	1	20.1	6.0%	6.6%
			2	20.3	6.9%	9.2%
		3	1	19.1	5.5%	8.0%
			2	18.9	8.5%	11.0%
2 A	80	1	1	21.8	5.5%	9.9%
			2	21.8	3.9%	7.5%
		2	1	22.6	5.6%	8.7%
			2	23.5	9.0%	10.3%
		3	1	23.9	7.0%	10.2%
			2	23.2	5.8%	8.9%
3 A	80	1	1	28.8	3.8%	7.8%
			2	30.7	4.3%	9.6%
		2	1	31.0	6.8%	8.0%
			2	31.4	4.3%	6.1%
		3	1	31.5	4.5%	6.4%
			2	32.2	4.0%	9.2%
4 A	80	1	1	49.3	3.9%	7.4%
			2	52.6	4.1%	6.7%
		2	1	50.8	5.6%	10.0%
			2	51.7	4.7%	5.9%
		3	1	52.6	4.6%	4.8%
			2	55.0	5.5%	6.1%
5 A	80	1	1	68.4	4.0%	7.6%
			2	73.2	3.7%	7.5%
		2	1	74.8	4.3%	8.2%
			2	75.9	4.6%	6.4%
		3	1	75.1	4.4%	7.9%
			2	76.3	4.9%	6.2%
7 A	80	1	1	115.9	4.2%	5.9%
			2	121.1	3.6%	7.0%
		2	1	123.2	4.3%	10.2%
			2	124.0	4.2%	6.4%
		3	1	127.0	4.8%	10.1%
			2	129.5	3.2%	5.6%
Low Control	80	1	1	23.7	9.4%	10.9%
			2	23.8	5.1%	11.5%
		2	1	20.0	6.0%	7.5%
			2	18.6	5.8%	8.5%
		3	1	20.3	8.3%	9.7%
			2	20.1	8.3%	10.0%
High Control	80	1	1	61.2	6.3%	6.4%
			2	58.8	4.5%	8.9%
		2	1	50.3	6.3%	8.1%
			2	50.2	5.9%	8.4%
		3	1	52.2	7.7%	9.2%
			2	50.8	5.8%	8.5%

12.4 Limit of Blank

In a representative study, Limit of Blank determinations were performed using two low-level holotranscobalamin samples and two reagent lots (120 replicates per reagent lot). The limit of blank of the BIOHIT Active B12 (HoloTC) EIA was found to be 4.9 pmol/L (rounded to 1 decimal place).

12.5 Limit of Detection

In a representative study, Limit of Detection determinations were performed using five low-level holotranscobalamin samples and two reagent lots (120 replicates per reagent lot). The limit of blank of the BIOHIT Active B12 (HoloTC) EIA was found to be 8.1 pmol/L (rounded up to 1 decimal place).

12.6 Limit of Quantitation

Limit of quantitation determinations were performed using five low-level holotranscobalamin samples and two reagent lots (120 replicates per reagent lot). The limit of quantification of the BIOHIT Active B12 (HoloTC) EIA was found to be 8.3 pmol/L (rounded to 1 decimal place).

12.7 High Dose Hook

High dose hook is a phenomenon whereby very high-level specimens may read within the dynamic range of the assay. For the BIOHIT Active B12 (HoloTC) EIA, no high dose hook effect was detected with two samples with a concentration of approximately 419 and 2236 pmol/L.

12.8 Cross-reactivity

The BIOHIT Active B12 (HoloTC) is designed to have a maximum deviation in holotranscobalamin concentration of ≤10% in the presence of apotranscobalamin or haptocorrin.

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2[12]. Three samples with holotranscobalamin levels across the assay range were supplemented with 500 pmol/L apotranscobalamin or 5000 pmol/L haptocorrin. The maximum deviation in holotranscobalamin concentration ranged from -5% to 1%.

12.9 Interference

The BIOHIT Active B12 (HoloTC) is designed to have a maximum deviation in holotranscobalamin concentration of ≤10% in the presence of potentially interfering compounds.

A study was performed based on guidance from the Clinical and Laboratory Standards Institute (CLSI) document EP7-A2[12]. Samples with holotranscobalamin levels across the assay range were supplemented with the potentially interfering compounds listed in Table 4. below. The maximum deviation in holotranscobalamin concentration ranged from –10% to 8%.

Table 4. List of tested interfering compounds.

Potential Interfering Substance	No interference found up to the following concentration
Hemoglobin	500 mg/dL
Bilirubin	30 mg/dL
Triglyceride (Intralipid Solution)	3000 mg/dL
Rheumatoid Factor	7500 IU/dL
Total Protein	9000 mg/dL

13. WARRANTY

Biohit shall remedy all defects discovered in any Product (the “Defective Product”) that result from unsuitable materials or negligent workmanship and which prevent the mechanical functioning or intended use of the Products including, but not limited to, the functions specified in Biohit’s specifications for the Products. ANY WARRANTLY WILL, HOWEVER, BE DEEMED AS VOID IF FAULT IS FOUND TO HAVE BEEN CAUSED BY MALTREATMENT, MISUSE, ACCIDENTAL DAMAGE, INCORRECT STORAGE OR USE OF THE PRODUCTS FOR OPERATIONS OUTSIDE THEIR SPECIFIED LIMITATIONS OR OUTSIDE THEIR SPECIFICATIONS, CONTRARY TO THE INSTRUCITONS GIVEN IN THE INSTRUCTION MANUAL. The period of this warranty is defined in the instruction manual of the Products and will commence form the date the relevant Product is shipped by Biohit. This Biohit Diagnostic kit has been manufactured according to ISO 9001 / ISO 13485 quality management protocols. In case of interpretation disputes the English text applies.

14. ORDERING INFORMATION

BIOHIT Active B12 (HoloTC) REF 602290

15. DATE OF ISSUE

BIOHIT Active B12 (HoloTC) Version 03, 05-2022

16. REFERENCES

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IVD	For *in vitro* diagnostic use	CONTROL LOW	Low Control
REF	Catalogue number	CONTROL HIGH	High Control
LOT	Batch code	CONJ	Conjugate
Σ 96	96 tests	SUBS	Substrate
	Caution	STOP	Stop Solution
	Consult instructions for use	PRE	Pre-Treatment
	Protect from Light	WASH 8x	Washing Buffer Concentrate (8x)
	Use by	BLANK	Blank
+2 +8°C	Store at 2-8°C	CAL 1-5	Calibrators 1 to 5
2	Do not re-use		Corrosive
			Harmful

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