Instruction For Use

【Product Name】

Vitamin B12 (eCLIA)

【Order Information】

REF NO.	Package Size
0320500207	50T
0320500208	100T
0320500203	2×50T
0320500204	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of Vitamin B12 in human serum and plasma.

Vitamin B12, also known as cobalamin, is a corrin family compound with four pyrrole rings around a cobalt atom[1]. As a complex organometallic compound, VB12 is mainly in the form of methylcobalamin in serum and 5' deoxyadenosylcobalamin in cells, which is converted to cyanocobalamin in the presence of cyanide[2]. Different forms of VB12 can act as coenzyme that catalyzes two important metabolic processes. Methylcobalamin can catalyze the conversion of homocysteine to methionine, and 5' deoxyadenosylcobalamin can catalyze the conversion of methylmalonyl coA to succinyl coA[3]. While, cyanocobalamin can be used as a reference compound for quantitative determination of vitamin B12 in serum.

Vitamin B12 is mainly obtained from animal diets such as meat, eggs and milk. Its absorption needs to form a complex with an intrinsic factor secreted by the cells of the gastric mucosa wall, and then be absorbed into the blood circulation in the ileum. Most vitamin B12 is stored in the liver and is transferred to cobalamin when needed and released into the plasma[2].

Lack of vitamin B12 not only damages nerve function, such as demyelination caused by abnormal methylation, which may seriously cause permanent damage, but also increases the risk of cardiovascular and cerebrovascular diseases[3]. In addition, vitamin B12 deficiency can hinder the synthesis of folic acid, affecting DNA synthesis, which in turn can affect the development and maturation of red blood cells, causing megaloblastic anemia[4].Vitamin B12 deficiency is mainly due to the lack of intrinsic factor. Insufficient vitamin B12 intake, gastrectomy, intestinal diseases, malabsorption, and cobalamin protein deficiency can also contribute to vitamin B12 deficiency.4 Studies have shown that the incidence of vitamin B12 deficiency increases with age[5].

【Test Principle】

Competition principle. Total duration of assay: 27 minutes

- 1st incubation: By incubating 50 µL of sample with pretreatment reagent 1, bound vitamin B12 is released from the vitamin B12 binding protein.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B12-binding protein complex is formed.
- 3rd incubation: After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied. A complex consisting of the ruthenylated intrinsic factor and the biotinylated Vitamin B12 is formed and becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrumentspecifically generated by 2 point calibration and a master curve provided via the reagent barcode.

【Main Component】

The reagent pack consists of MB, RA, RB, RD, calibrators and control materials (Optional). Different lots cannot be used at the same time or mixed up together.

Component	Ingredients	Volume (2×50T)	Volume (1×50T)	Volume (1×100T)	Volume (2×100T)
MB	Streptavidin-coated microparticles, 0.75 mg/mL: 0.1M PBS, 0.05% ProClin™ 300	2×1.8 mL	1×1.8 mL	1×3.5 mL	2×3.5 mL
RB	Vitamin B12~ Biotin: 0.05M phosphate buffer, 0.05% ProClin™ 300	2×2.0 mL	1×2.0 mL	1×4.0 mL	2×4.0 mL
RA	Intrinsic factor ~$Ru(bpy)_3^{2+}$: 0.05M phosphate buffer, 0.05% ProClin™ 300	2×3.8 mL	1×3.8 mL	1×7.5 mL	2×7.5 mL
RD	40g/L Sodium Hydroxide	2×1.0 mL	1×1.0 mL	1×2.0 mL	2×2.0 mL
Calibrators	Vitamin B12, 0.05M phosphate buffer, 0.05% ProClin™ 300, lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	Vitamin B12, 0.05M phosphate buffer, 0.05% ProClin™ 300, lyophilized	High: 1×2.0 mL Low: 1×2.0 mL			

Traceability: This kit can be traced to 1st International Standard for vitamin B12 from the National Institute for Biological Standards and Control (NIBSC) code 03/178.

See the control material value sheet for the target values and ranges of control materials.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic).

Additional materials for Automated ECL Immunoassay Analyzers eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzers eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】

Store at 2°C-8°C.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Damaged, expired or contaminated reagents should be discarded.

Stability of the reagents	
Unopened at 2°C~8°C	18 months
Store at 2°C~8°C after opening	8 weeks
on the analyzers at 2°C~15°C	8 weeks

The calibrators and control materials are stable up to the stated expiration date.

Stability of the calibrators and control materials	
Lyophilized, unopened at 2°C~8°C	18 months
Reconstituted at 2°C~8°C	7 days
Reconstituted on the analyzers at 18 °C-28 °C	8 hours
Reconstituted at -15°C or lower than -15°C	3 months (1 freeze/thaw cycle possible)

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, heparin sodium, EDTA-K_3 and EDTA-K_2 anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture, after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended.

Samples can be stored for 2 hours at 18°C-28 °C, 48 hours at 2°C-8 °C, 2 months at -15°C or lower than -15°C. The samples may be frozen once.

The samples should be centrifugated before test if samples are deposited and frozen.

Ensure the samples and calibrators are at 18°C-28 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Vitamin B12 test according to the operational manual.

Put the Vitamin B12 rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 minutes before testing.

Recommended ambient temperature:10~30°C; Relative humidity: ≤ 80%.

The sample volume required for each vitamin B12 test is 50 µL

Handing of lyophilized calibrators and control materials

Carefully dissolve the contents of calibrator by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed at room temperature for at least 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators to the additional plastic bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15°C or lower than -15°C.

Carefully dissolve the contents of control materials by adding exactly 2.0 mL of distilled or deionized water and allow to stand closed at room temperature for at least 15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted control materials to the additional plastic bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -15°C or lower than -15°C.

Calibration

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification reagent card(refer to instrument user manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.
Recalibration is recommended when:
(1) The reagents of different lot are used;
(2) The same lot of reagents were used on the analyzer beyond 4 weeks;
(3) Quality control findings outside the defined limits.
(4) The Buffer of different lots are used.

Quality control

For quality control, use Vitamin B12 Control Materials.
In addition, other suitable control material can be used.
Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
If necessary, repeat the measurement of the samples concerned.
Follow the applicable government regulations and local guidelines for quality control.
Please make sure that the correct values are used.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is pg/mL or pmol/L: pmol/L × 1.36 = pg/mL, pg/mL × 0.738 = pmol/L.

Specimen dilution

Samples with Vitamin B12 concentrations above the detection range can be diluted with sample dilution. It is recommended to dilute at 1:2 (by instrumental automatic dilution or manual dilution). If diluted by hand, the result should be multiplied by the dilution multiple. If the instrument automatically dilutes, the instrument automatically calculates the result.

【Biological reference interval】

According to serum test results of 240 healthy human (male 120, female 120) , the reference range is 197-771 pg/mL.
Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges.

【Result Interpretation】

For explaining the result, the patient's overall clinical manifestation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.
When the sample containing bilirubin ≤ 65 mg/dL ,triglyceride ≤ 1500 mg/dL, biotin ≤ 50 ng/mL, or hemoglobin ≤ 0.04 g/dL, interference deviation to the test result lies within ±10%. When the concentration of rheumatoid factor in the sample ≤ 1500 IU/mL, the relative recovery of the test results is between 90%- 110%.
Samples with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. High total protein samples may lead to the formation of protein gel in the assay cup, which may cause an abnormally high result. The critical total protein concentration is dependent upon the individual sample composition.

【Measuring Range】

The measuring range of the kit is 50-2000 pg/mL. If the Vitamin B12 concentration in the sample is lower than the lower limit of detection, the result is reported as < 50 pg/mL. If the Vitamin B12 concentration in the sample is higher than the upper limit of detection, the result is reported as > 2000 pg/mL (2 × diluted sample is reported as > 4000 pg/mL).

【Analytical Performance】

Lower limit of measurement

Limit of Blank = 50 pg/mL
Limit of Detection = 100 pg/mL.

Accuracy

The relative deviation between the measured value and the theoretical value of national or international standard substances should be within ±15%.
Or The relative deviation between the measured value and the theoretical value of the standard traceability accuracy control products should be within ±10%.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 100-2000 pg/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is no more than 8.0%.

Between-lot precision

The coefficient of variation (CV) is no more than 10.0%.

Analytical specificity

The following cross-reacting substance was found, tested with vitamin B12 concentrations of 200 pg/mL and 600 pg/mL .

Analog	Concentration	Cross-reactivity (%)
Dicyanocobinamide	200 ng/mL	≤ 0.005

Method Comparison

A comparison of the Lifotronic Vitamin B12 (eCLIA) (y) with the Roche Elecsys Vitamin B12 Method (x) using clinical samples gave the following correlations:
Number of serum samples measured: 113
Linear regression: y=1.0019 x - 13.683,
r=0.983
The sample concentrations were between 183.8- 1695 pg/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
Exercise the normal precautions required for handling all laboratory reagents.
All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.
Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/ spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant. The abandoned reagents should be deal with in compliance with local regulations.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limitation		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
P/N	Material code	REF	Catalogue number
	Corrosive chemical identification		Warning
	Date of manufacture	UDI	Unique device identifier

【Reference】

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5. Pennypacker LC, Allen RH, Kelly JP, et al. High prevalence of cobalamin deficiency in elderly outpatients[J]. Journal of the American Geriatrics Society, 1992, 40(12):1197.

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