

# Vitamin B12

Vitamin B<sub>12</sub>

04745736 190

100 tests

cobas®

- Indicates analyzers on which the kit can be used

Elecsys 2010	MODULAR ANALYTICS E170	cobas e 411	cobas e 601
•	•	•	•

## English

### Intended use

Binding assay for the in vitro quantitative determination of vitamin B<sub>12</sub> in human serum and plasma.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.

### Summary

Nutritional and macrocytic anemias can be caused by a deficiency of vitamin B<sub>12</sub>. This deficiency can result from diets devoid of meat and bacterial products, from alcoholism, or from structural/functional damage to digestive or absorptive processes (forms of pernicious anemia). Malabsorption is the major cause of this deficiency through pancreatic deficiency, gastric atrophy or gastrectomy, intestinal damage, loss of intestinal vitamin B<sub>12</sub> binding protein (intrinsic factor), production of autoantibodies directed against intrinsic factor, or related causes.<sup>1</sup> This vitamin is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies will lead to megaloblastic anemia, and vitamin B<sub>12</sub> deficiency results in irreversible central nervous system degeneration. Vitamin B<sub>12</sub> or folate are both of diagnostic importance for the recognition of vitamin B<sub>12</sub> or folate deficiency, especially in the context of the differential diagnosis of megaloblastic anemia. Radioassays were first reported for vitamin B<sub>12</sub> in 1961.<sup>3,4</sup> All utilize <sup>57</sup>Co-cyanocobalamin radiolabeled tracers and intrinsic factor for binding vitamin B<sub>12</sub>. The various commercial assays differ in their free versus bound separation techniques and choice of specimen pretreatment. The presence of endogenous serum binding proteins for cyanocobalamin (transcobalamins including R-protein) and of immunoglobulins directed against intrinsic factor require that specimens are either boiled or treated at an alkaline pH to release the vitamin B<sub>12</sub> and destroy the binding proteins. In the late 1970's, radioassays using serum binding proteins or partially purified intrinsic factor measured levels of vitamin B<sub>12</sub> which exceeded those determined by microbiological methods. This was caused by the presence of the serum binding protein or R-proteins in the assay. R-protein specificity is poor compared to that of intrinsic factor and vitamin B<sub>12</sub> analogs were being measured in addition to vitamin B<sub>12</sub> itself.<sup>5,6,7,8</sup> Since that time, recommendations have been established for the use of highly purified intrinsic factor throughout the industry. The Elecsys Vitamin B<sub>12</sub> assay employs a competitive test principle using intrinsic factor specific for vitamin B<sub>12</sub>. Vitamin B<sub>12</sub> in the sample competes with the added vitamin B<sub>12</sub> labeled with biotin for the binding sites on the ruthenium-labeled intrinsic factor complex<sup>a</sup>.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)<sub>3</sub><sup>2+</sup>)

### Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating the sample (15 µL) with the vitamin B<sub>12</sub> pretreatment 1 and pretreatment 2, bound vitamin B<sub>12</sub> is released.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled intrinsic factor, a vitamin B<sub>12</sub>-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample.
- 3rd incubation: After addition of streptavidin-coated microparticles and vitamin B<sub>12</sub> labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor-vitamin B<sub>12</sub> biotin complex. The entire complex 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 ProCell. 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 instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

### Reagents - working solutions

- PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:  
Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.
- PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL:  
Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.
- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:  
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Intrinsic factor~Ru(bpy)<sub>3</sub><sup>2+</sup> (gray cap), 1 bottle, 10 mL:  
Ruthenium labeled porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.
- R2 Vitamin B<sub>12</sub>-biotin (black cap), 1 bottle, 8.5 mL:  
Biotinylated vitamin B<sub>12</sub> 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

### Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

This kit contains components classified as follows according to the European directive 88/379/EEC:



PT2: C - CORROSIVE, R 34, S 26, S 37/39 (sodium hydroxide)  
Causes burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective gloves and eye/face protection.



PT2: Xn - HARMFUL, R 20/21/22, S 45 (sodium cyanide)  
Harmful by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-621-7590, USA: +1-800-428-2336

All human material should be considered potentially infectious.

All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.<sup>9,10</sup>

Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

### Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in via the respective reagent barcodes.

### Storage and stability

Store at 2-8°C.

Store the Elecsys Vitamin B<sub>12</sub> reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

unopened at 2-8°C	up to the stated expiration date
after opening at 2-8°C	12 weeks
on Elecsys 2010 and cobas e 411	5 weeks
on MODULAR ANALYTICS E170 and cobas e 601	5 weeks



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### Specimen collection and preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel.

Na-heparin and K<sub>3</sub>-EDTA plasma. When sodium citrate, sodium fluoride/potassium oxalate are used, the values obtained are by 23% lower as compared to serum.

Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within  $\pm 2 \times$  analytical sensitivity (LDL) + coefficient of correlation  $> 0.95$ . Stable for 2 days at 2-8°C, 2 months at -20°C. Freeze once only. Protect from light.

Stability of serum obtained with separating tubes: 24 hours at 2-8°C (note the data provided by the tube manufacturer).

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.

Vitamin B<sub>12</sub> determinations should be performed on serum or plasma samples from fasting patients.

*Note:* Samples with extremely high total protein concentrations (e.g. patients suffering from Waldenström's macroglobulinemia) are not suitable for use in this assay, since they may lead to the formation of protein gel in the assay cup. Processing protein gel may cause a run abort. The critical protein concentration is dependent upon the individual sample composition. The formation of protein gel was seen in samples (spiked with human IgG or human serum albumin) having a total protein concentration  $> 160$  g/L.

Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.

Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

### Materials provided

See "Reagents - working solutions" section for reagents.

### Materials required (but not provided)

- Cat. No. 04572459, Vitamin B<sub>12</sub> CalSet II, for 4 x 1 mL
- Cat. No. 04415299, PreciControl Anemia, for 2 x 2 mL each of PreciControl Anemia 1, 2 and 3
- Cat. No. 11732277, Diluent Universal, 2 x 16 mL sample diluent or Cat. No. 03183971, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- Cat. No. 11662988, ProCell, 6 x 380 mL system buffer
- Cat. No. 11662970, CleanCell, 6 x 380 mL measuring cell cleaning solution
- Cat. No. 11930346, Elecsys SysWash, 1 x 500 mL washwater additive
- Cat. No. 11933159, Adapter for SysClean
- Cat. No. 11706802, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- Cat. No. 11706799, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170 and **cobas e** 601 analyzers:

- Cat. No. 04880340, ProCell M, 2 x 2 L system buffer
- Cat. No. 04880293, CleanCell M, 2 x 2 L measuring cell cleaning solution
- Cat. No. 12135027, CleanCell M, 1 x 2 L measuring cell cleaning solution (for USA)
- Cat. No. 03023141, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- Cat. No. 03005712, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- Cat. No. 03004899, PreClean M, 5 x 600 mL detection cleaning solution
- Cat. No. 12102137, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags

- Cat. No. 03023150, WasteLiner, waste bags
- Cat. No. 03027651, SysClean Adapter M

Accessories for all analyzers:

- Cat. No. 11298500, Elecsys SysClean, 5 x 100 mL system cleaning solution

Only available in the USA:

- Cat. No. 04836693, Elecsys Vitamin B<sub>12</sub> CalCheck, 3 concentration ranges

### Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers: Bring the cooled reagent to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.

### Calibration

Traceability: This method has been standardized against the Elecsys Vitamin B<sub>12</sub> assay (Cat. No. 11820753).

Every Elecsys Vitamin B<sub>12</sub> reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Vitamin B<sub>12</sub> CalSet II.

*Calibration frequency:* Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. if quality control findings are outside the specified limits

### Quality control

For quality control, use Elecsys PreciControl Anemia 1, 2 and 3.

Other suitable control material can be used in addition.

Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every 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 limits.

### Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or pg/mL).

Conversion factors:  $\text{pmol/L} \times 1.36 = \text{pg/mL}$   
 $\text{pg/mL} \times 0.738 = \text{pmol/L}$

### Limitations - interference

The assay is unaffected by icterus (bilirubin  $< 1112$   $\mu\text{mol/L}$  or  $< 65$  mg/dL), hemolysis (Hb  $< 0.621$  mmol/L or  $< 1.0$  g/dL), lipemia (triglycerides  $< 17.1$  mmol/L or  $< 1500$  mg/dL), and biotin  $< 205$  nmol/L or  $< 50$  ng/mL.

Criterion: Recovery within  $\pm 10\%$  of initial value.

In patients receiving therapy with high biotin doses (i.e.  $> 5$  mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

In vitro tests were performed on 54 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.



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### Measuring range

22-1476 pmol/L or 30-2000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 22 pmol/L or < 30 pg/mL. Values above the measuring range are reported as > 1476 pmol/L or > 2000 pg/mL.

### Dilution

Samples with vitamin B<sub>12</sub> concentrations above the measuring range can be manually diluted 1:2 with Elecsys Diluent Universal. The concentration of the diluted sample must be > 738 pmol/L or > 1000 pg/mL. After manual dilution, multiply the results by the dilution factor 2.

*Note:* Sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range. As Elecsys Diluent Universal may contain low levels of endogenous vitamin B<sub>12</sub>, it is recommended that linearity studies be performed using a known low analyte-containing serum pool. Samples outside the measuring range can be diluted 1:2 with Elecsys Diluent Universal; the effect of endogenous vitamin B<sub>12</sub> concentration is insignificant at these levels.

### Expected values

Because differences may exist with respect to population and dietary status, it is recommended that normal ranges be determined by each laboratory over a suitable period of time and in a statistically significant number of assays before clinical significance is attached to the results of these tests.

The values shown below were obtained in a study performed 2005 in the USA and Germany:

Region	N	Median		Range (2.5 <sup>th</sup> -97.5 <sup>th</sup> percentile)	
		pmol/L	pg/mL	pmol/L	pg/mL
Europe	291	263	357	141-489	191-663
USA	178	342	463	156-698	211-946

These values should only be used as a guideline.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

### Precision

Reproducibility was determined using Elecsys reagents, pooled human sera, and controls in a modified protocol (EP5-A) of the NCCLS (National Committee for Clinical Laboratory Standards):<sup>11</sup> 6 times daily for 10 days (n = 60); within-run precision on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and cobas e 411 analyzers								
Sample	Mean		Within-run precision			Total precision		
	pmol/L	pg/mL	SD pmol/L	SD pg/mL	CV %	SD pmol/L	SD pg/mL	CV %
HS <sup>b</sup> 1	171	232	14.9	20.2	8.7	16.2	22.0	9.5
HS 2	604	818	26.8	36.3	4.4	30.6	41.5	5.1
HS 3	919	1245	27.3	37.0	3.0	34.1	46.2	3.7
PCA <sup>c</sup> 1	167	226	13.9	18.8	8.3	13.4	18.1	8.0
PCA 2	450	610	17.8	24.1	4.0	23.3	31.6	5.2
PCA 3	1010	1369	31.4	42.5	3.1	34.7	47.0	3.4

b) HS = human serum

c) PCA = PreciControl Anemia

MODULAR ANALYTICS E170 and cobas e 601 analyzers										
Sample	Within-run precision					Total precision				
	Mean		SD		CV	Mean		SD		CV
	pmol/L	pg/mL	pmol/L	pg/mL	%	pmol/L	pg/mL	pmol/L	pg/mL	%
HS 1	197	267	2.4	3.3	1.2	199	269	16.6	22.5	8.4
HS 2	655	887	19.2	26.0	2.9	630	853	21.0	28.3	3.3
HS 3	965	1308	14.8	20.0	1.5	931	1261	22.8	31.0	2.5
PCA 1	227	308	3.8	5.1	1.7	176	239	13.3	18.0	7.5
PCA 2	524	710	5.2	7.1	1.0	486	658	18.1	24.5	3.7
PCA 3	1125	1524	9.6	13	0.8	1044	1415	24.6	33.3	2.4

### Analytical sensitivity (lower detection limit)

22 pmol/L or 30 pg/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, within-run precision, n = 21).

### Method comparison

A comparison of the Elecsys Vitamin B<sub>12</sub> assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B<sub>12</sub> CalSet; x) and the Elecsys Vitamin B<sub>12</sub> assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B<sub>12</sub> CalSet II; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 101

Passing/Bablok <sup>12</sup>	Linear regression
y = 0.982x - 0.018	y = 0.968x + 5.77
τ = 0.977	r = 0.999

The sample concentrations were between approx. 49 and 1691 pg/mL (approx. 36 and 1248 pmol/L).

A comparison of the Elecsys Vitamin B<sub>12</sub> assay (MODULAR ANALYTICS E170 analyzer; calibrated with Elecsys Vitamin B<sub>12</sub> CalSet II; x) and the Elecsys Vitamin B<sub>12</sub> assay (Elecsys 2010 analyzer; calibrated with Elecsys Vitamin B<sub>12</sub> CalSet II; y) using clinical samples gave the following correlations (pg/mL):

Number of samples measured: 100

Passing/Bablok <sup>12</sup>	Linear regression
y = 0.997x - 4.17	y = 0.978x - 0.479
τ = 0.930	r = 0.994

The sample concentrations were between approx. 55 and 1609 pg/mL (approx. 41 and 1187 pmol/L).

### Analytical specificity

The following cross-reactivity was found:

Cobinamide dicyanide	200 ng/mL	0.024%
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### References

- Herbert V. Drugs effective in megaloblastic anemias. In Goodman LS and Gilman A (eds): The Pharmacological Basis of Therapeutics, 5th Ed. MacMillan Co. 1975;324-1349.
- Ross GIM. Vitamin B<sub>12</sub> in body fluids. Nature 1950;166:270.
- Barakat RM, Ekins P. Assay of vitamin B<sub>12</sub> in blood: A simple method. Lancet 1961;i:25-26.
- Rothenberg SP. Assay of serum vitamin B<sub>12</sub> concentration using Co57-B12 and intrinsic factor. Proceedings of the Society for Experimental Biology and Medicine 1961;108:45-48.
- Rothenberg SP, DaCosta M, Rosenberg BS. A radioassay for serum folate: Use of a two-phase sequential incubation, ligand-binding system. New Eng J Med 1972;285(25):1335-1339.
- Gutcho S, Mansbach L. Simultaneous radioassay of serum vitamin B<sub>12</sub> and folic acid. Clin Chem 1977;23:1609-1614.
- Kolhouse JF, Kondo H, Allen NC, Podell AB, Allen RH. Cobalamin analogues are present in human plasma and can mask cobalamin deficiency because current radioisotope dilution assays are not specific for true cobalamin. New Eng J Med 1978;299(15):785-792.
- BIO RAD Quantaphase B-12/Folate Radioassay Instruction Manual, März 1995.
- Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register. July 1, 2001;17:260-273.
- Council Directive (2000/54/EC). Official Journal of the European Communities No. L262 from Oct. 17, 2000.
- NCCLS Proposed Standard: PSLA-12, Guidelines for Evaluating a B<sub>12</sub> (COBALAMIN) Assay, March 1980.
- Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.



# Vitamin B12

Vitamin B<sub>12</sub>

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