

Folate III

Folate

cobas®

REF	Σ	SYSTEM
04476433 190	100	Elecsys 2010 MODULAR ANALYTICS E170 cobas e 411 cobas e 601 cobas e 602

English

Intended use

Binding assay for the in vitro quantitative determination of folate in human serum and plasma.

The binding assay is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Nutritional and macrocytic anemias can be caused by a deficiency of folate. This deficiency can result from diets devoid of raw fruits, vegetables or other foods rich in folic acid, as may be the case with chronic alcoholics, drug addicts, the elderly or persons of low socioeconomic status, etc. In addition, low serum folate during pregnancy has been associated with neural tube defects in the fetus.¹ Dietary deficiency and malabsorption are the major causes of folate deficiency in humans.² Folate is necessary for normal metabolism, DNA synthesis and red blood cell regeneration. Untreated deficiencies may lead to megaloblastic anemia.

Since a deficiency of either vitamin B₁₂ or folate can cause megaloblastic anemia, it is advisable to determine the concentration of both vitamin B₁₂ and folate in order to properly diagnose the etiology of anemia. Radioassays were first reported for folate in 1973.^{3,4,5,6}

The majority utilize ¹²⁵I-folate radiolabeled tracers and natural binding proteins (milk binding protein, folate binding protein). The various commercial assays differ in their free versus bound separation techniques and choice of specimen pretreatment.

The Elecsys Folate III assay employs a competitive test principle using natural folate binding protein (FBP) specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on FBP (labeled with ruthenium complex^a).

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating 25 µL of sample with the folate pretreatment reagents 1 and 2, bound folate is released from endogenous folate binding proteins.
- 2nd incubation: By incubating the pretreated sample with the ruthenium labeled folate binding protein, a folate 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 folate labeled with biotin, the unbound sites of the ruthenium labeled folate binding protein become occupied, with formation of a ruthenium labeled folate binding protein-folate 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/ProCell M. 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

The reagent rackpack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as Fol III.

PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL:

Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 5 mL:

Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Folate binding protein~Ru(bpy)₃²⁺ (gray cap), 1 bottle, 9 mL:

Ruthenium labeled folate binding protein 75 µg/L; human serum albumin (stabilizer); borate/phosphate/citrate buffer 70 mmol/L, pH 5.5; preservative.

R2 Folate~biotin (black cap), 1 bottle, 8 mL:

Biotinylated folate 17 µg/L; biotin 120 µg/L; human serum albumin (stabilizer); borate buffer 100 mmol/L, pH 9.0; 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 in accordance with the Regulation (EC) No. 1272/2008:



Danger

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

Prevention:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P301 + P330 IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. + P331

P303 + P361 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. + P353

P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER or doctor/physician. + P310

P305 + P351 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. + P338

P390 Absorb spillage to prevent material damage.

Product safety labeling primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of

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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 handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{7,8}

Avoid foam formation in 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 from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys 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	8 weeks
on the analyzers	2 weeks

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.

Li-heparin plasma. Li-heparin plasma tubes containing separating gel can be used.

Criterion: Method comparison serum versus Li-heparin plasma, slope 0.9-1.1 + intercept within $\pm 2 \times$ analytical sensitivity (LDL/LoB), coefficient of correlation ≥ 0.95 .

Serum: Stable for 2 hours at 20-25 °C, 2 days at 2-8 °C, 1 month at -20 °C. Freeze only once. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

Li-heparin plasma: Stable for 2 hours at 20-25 °C, 2 days at 2-8 °C. Do not freeze samples containing Li-heparin. Protect from light. Store the samples at 2-8 °C if they cannot be measured immediately.

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.

Samples should not subsequently be altered with additives (biocides, anti-oxidants or substances possibly changing the pH of the sample) in order to avoid erroneous folate recovery.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

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

Note: Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay. Samples for folate determinations should be collected from fasting persons.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- [REF] 04874072190, Folate III CalSet, for 4 x 1.0 mL

- [REF] 05618860190, PreciControl Varia, for 2 x 3 mL each of PreciControl Varia 1 and 2

- [REF] 11732277122, Diluent Universal, 2 x 16 mL sample diluent or [REF] 03183971122, 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:

- [REF] 11662988122, ProCell, 6 x 380 mL system buffer
- [REF] 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- [REF] 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- [REF] 11933159001, Adapter for SysClean
- [REF] 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- [REF] 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- [REF] 04880340190, ProCell M, 2 x 2 L system buffer
- [REF] 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- [REF] 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- [REF] 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- [REF] 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- [REF] 03023150001, WasteLiner, waste bags
- [REF] 03027651001, SysClean Adapter M

Accessories for all analyzers:

- [REF] 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

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

Resuspension of the microparticles takes place automatically prior to 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, **cobas e** 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. 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 Folate II assay ([REF] 03253678).

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

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:

- 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. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Varia.

In addition, other suitable control material can be used.

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Controls 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.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

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

Conversion factors:

$$\text{nmol/L} \times 0.44 = \text{ng/mL}$$

$$\text{ng/mL} \times 2.27 = \text{nmol/L}$$

Limitations - interference

The assay is unaffected by icterus (bilirubin < 564 µmol/L or < 33 mg/dL), lipemia (Intralipid < 1500 mg/dL), biotin (< 86.1 nmol/L or < 21 ng/mL), IgG < 16 g/L and IgA < 4.0 g/L.

Criterion: Recovery within ± 10 % of initial value with samples > 5 ng/mL and ≤ ± 0.5 ng/mL with samples ≤ 5 ng/mL.

Hemolysis may significantly increase folate values due to high concentrations of folate in red blood cells. Therefore, hemolyzed samples are not suitable for use in this assay.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1000 IU/mL.

In vitro tests were performed on 18 commonly used pharmaceuticals and in addition on human erythropoietin. No interference with the assay was found.

It is contraindicated to measure samples of patients receiving therapy with certain pharmaceuticals, e.g. methotrexate or leucovorin, because of the cross-reactivity of folate binding protein with these compounds.

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.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur. These effects are minimized by suitable test design.

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

Limits and ranges

Measuring range

0.640-20.0 ng/mL or 1.45-45.4 nmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.640 ng/mL (< 1.45 nmol/L). Values above the measuring range are reported as > 20.0 ng/mL (> 45.4 nmol/L).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: ≤ 0.640 ng/mL (≤ 1.45 nmol/L)

The lower detection limit represents the lowest 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, repeatability study, n = 21).

Dilution

Samples with folate concentrations above the measuring range can be diluted manually with Diluent Universal. The recommended dilution is 1:2. The concentration of the diluted sample must be > 10.0 ng/mL or > 22.7 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

Expected values

Referring to "The American Journal of Clinical Nutrition"⁹ serum folate (folic acid) values were found as follows:

Sex	Age years	N	Median		2.5 th -97.5 th percentile	
			nmol/L	ng/mL	nmol/L	ng/mL
Both	all	23345	29.5	13.0	10.4-78.9	4.6-34.8
Male	all	11387	27.9	12.3	10.2-73.0	4.5-32.2
Female	all	11958	30.1	13.3	10.9-84.5	4.8-37.3
Both	4-11	3595	39.0	17.2	19.5-85.4	8.6-37.7
Both	12-19	6390	27.4	12.1	11.3-61.6	5.0-27.2
Both	20-59	8689	26.3	11.6	10.0-70.2	4.4-31.0
Both	≥ 60	4671	37.6	16.6	12.7-103.8	5.6-45.8

These values were obtained in the USA during the National Health and Nutrition Examination Survey (NHANES), 1999-2004.

The values shown below were performed on samples from an apparently healthy population, using the Elecsys Folate III assay.

Europe: The calculation is based on 290 sera (96 men, 194 women). The age range was between 18 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values.

Australia: The calculation is based on 345 sera (217 men, 128 women). The age range was between 18 and 65 years. Pregnant women were excluded. The reference population was selected according to normal homocysteine values.

Country	N	Median		2.5 th -97.5 th percentile	
		nmol/L	ng/mL	nmol/L	ng/mL
Europe	290	21.7	9.57	10.4-42.4	4.6-18.7
Australia	345	23.9	10.6	12.0-43.9	5.3-19.3

Please note: These values should only be used as a guideline.

It should be taken into consideration that differences in the expected values may exist with respect to population and dietary status.

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

Folate deficient sample values

25 samples considered to be deficient^{b)} in serum folate concentration were assessed using the Elecsys Folate III assay. All samples were found to be below the 2.5th percentile as given in the table above.

^{b)} Folate deficiency was assessed by measurement of serum folate by two commercially available folate assays.

Specific performance data

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

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and cobas e 411 analyzers								
			Repeatability			Intermediate precision		
Sample	Mean		SD		CV	SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HS ^e 1	9.76	4.30	0.356	0.157	3.7	0.869	0.383	8.9
HS 2	14.0	6.17	0.622	0.274	4.4	1.01	0.444	7.2
HS 3	15.5	6.83	0.654	0.288	4.2	1.10	0.484	7.1
HS 4	35.6	15.7	1.41	0.620	4.0	2.17	0.956	6.1

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Elecsys 2010 and cobas e 411 analyzers								
		Repeatability			Intermediate precision			
Sample	Mean		SD		CV	SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
PC V ^d 1	9.19	4.05	0.533	0.235	5.8	0.867	0.382	9.4
PC V2	26.8	11.8	1.28	0.562	4.8	1.92	0.845	7.2

c) HS = human serum

d) PC V = PreciControl Varia

MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers								
		Repeatability			Intermediate precision			
Sample	Mean		SD		CV	SD		CV
	nmol/L	ng/mL	nmol/L	ng/mL	%	nmol/L	ng/mL	%
HS 1	9.99	4.40	0.341	0.150	3.4	0.400	0.176	4.0
HS 2	14.6	6.43	0.661	0.291	4.5	0.926	0.408	6.3
HS 3	16.3	7.19	0.556	0.245	3.4	0.824	0.363	5.0
HS 4	34.3	15.1	0.822	0.362	2.4	1.56	0.688	4.6
PC V1	8.88	3.91	0.449	0.198	5.1	0.624	0.275	7.0
PC V2	27.5	12.1	0.842	0.371	3.1	0.940	0.414	3.4

Method comparison

a) A comparison of the Elecsys Folate III assay (MODULAR ANALYTICS E170 analyzer; calibrated with Folate III CalSet; y) and the Elecsys Folate II assay (MODULAR ANALYTICS E170 analyzer; calibrated with Folate II CalSet II; x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 98

Passing/Bablok¹⁰ Linear regression
 $y = 1.11x + 0.317$ $y = 1.12x + 0.236$
 $r = 0.939$ $r = 0.994$

The sample concentrations were between approximately 1.96 and 14.6 ng/mL (approximately 4.45 and 33.1 nmol/L).

b) A comparison of the Elecsys Folate III assay on the MODULAR ANALYTICS E170 analyzer (y) with the Elecsys Folate III assay on the Elecsys 2010 analyzer (x) (both tests have been calibrated with Folate III CalSet) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 149

Passing/Bablok¹⁰ Linear regression
 $y = 0.956x + 0.129$ $y = 0.925x + 0.314$
 $r = 0.858$ $r = 0.976$

The sample concentrations were between approximately 3.10 and 14.6 ng/mL (approximately 7.04 and 33.1 nmol/L).

c) A comparison of the Elecsys Folate III assay on the MODULAR ANALYTICS E170 analyzer (y) with another commercially available assay (x) using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 170

Passing/Bablok¹⁰ Linear regression
 $y = 0.794x + 2.15$ $y = 0.645x + 2.95$
 $r = 0.673$ $r = 0.843$

The sample concentrations were between approximately 1.19 and 18.9 ng/mL (approximately 2.70 and 42.9 nmol/L).

Analytical specificity

The following cross-reactivities were found:

Aminopterin 2.7 %

Folinic acid 2.3 %

Amethopterin 2.3 %

Functional sensitivity

≤ 2.0 ng/mL (≤ 4.54 nmol/L)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

References

- 1 Rush D. Folate Supplements Prevent Recurrence of Neural Tube Defects, FDA Dietary Supplement Task Force. Nutrition Reviews 1992;50(1):22-28.
- 2 Herbert V. Drugs effective in megaloblastic anemias. In Goodman LS and Gilman A (eds): The Pharmacological Basis of Therapeutics, 5th Ed, MacMillan Co, 1975;1324-1349.
- 3 Dunn RT, Foster LB. Radioassay of serum Folate. Clin Chem 1973;19:1101-1105.
- 4 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.
- 5 Gutcho S, Mansbach L. Simultaneous radioassay of serum Folate and folic acid. Clin Chem 1977;23:1609-1614.
- 6 BIO RAD Quantaphase B-12/Folate Radioassay Instruction Manual. March 1995.
- 7 Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
- 8 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
- 9 Pfeiffer CM, Johnson CL, Jain RB, et al. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988-2004. Am J Clin Nutr 2007;86:718-727.
- 10 Bablok W, Passing H, Bender R, et al. A general regression procedure for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):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 Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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