Elecsys Folate III

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REF		Σ	SYSTEM
08324174190	08324174500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)	Application
FOL 3	10168	Folate serum/plasma
RBC 2	10169	Folate RBC application

Intended use

Binding assay for the in vitro quantitative determination of folate in human serum, plasma and erythrocytes (red blood cells, RBC). Folate measurements, performed with the Elecsys Folate III assay, are used as an aid in diagnosis and monitoring of folate imbalance.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Folate deficiency may be due to several clinical conditions such as decreased nutritional intake, poor absorption of ingested folate in the intestine, increased demand of folate (during physical activity or pregnancy), liver diseases, impaired folate metabolism due to genetic defects or due to drug interactions. Folate measurements are also used to aid in diagnosis of megaloblastic (macrocytic) anemia.

Folate belongs to the family of B-group vitamins composed of an aromatic pteridine ring linked through a methylene group to p-aminobenzoic acid and a glutamate residue. Folate (folic acid) is vital for normal cellular functions and plays an essential role in nucleic acid synthesis, methionine regeneration, shuttling and redox reactions of one-carbon-units required for normal metabolism and regulation.[1,2]

The folate metabolism can be exemplified as a cycle, where folate facilitates the transfer of one-carbon-units from one molecule to another required in various biochemical reactions: for example, tetrahydrofolate (THF) accepts a single carbon unit from serine, which is reduced in a number of steps to 5-methyltetrahydrofolate (5-MTHF). 5-MTHF gives its methyl group to homocysteine, which is - with involvement of methionine synthase and vitamin B12 - enzymatically converted to methionine. The resulting THF starts again the cycle of methyl group synthesis. From methionine, the methyl groups are transferred to S-adenosylmethionine (SAM).[3] SAM serves as a methyl group donor in several methylation reactions, like DNA, RNA and protein methylation.[1]

The methionine cycle is highly sensitive to folate deficiency: with a low folate status, the ability of the cell to re-methylate homocysteine is impaired and this results in increased homocysteine concentrations in plasma.[2]

Folate also plays an essential role in the synthesis of purine and pyrimidine precursors of nucleic acids. Altered distribution of methyl groups and impaired DNA synthesis play an essential role in the development of cancers. Abnormal folate status has also been linked with the development of diseases like cardiovascular diseases, neural tube defects, cleft lip and palate, late pregnancy complications, neurodegenerative and psychiatric disorders.[1,2]

Folate belongs to the group of essential vitamins, i.e. it cannot be synthesized by the human organism and therefore must be absorbed from diet. Primary sources of folates are green and leafy vegetables, sprouts, fruits, brewer's yeast and liver.[1,2]

In children, the demand of folate is particularly high during the period of rapid growth.[4] The normal infant requirement is 25-35 µg/day, and weight-based requirements are higher in children compared to adults due to the increased needs of folate to support growth.

In children, the normal range of RBC folate is 150-600 ng/mL.[5] and the RBC-folate cutoff value of < 151 ng/mL (< 340 nmol/L) indicates folate deficiency in all age groups, including children.[6,7]

Serum folate concentrations are higher in small children, and the level decreases with age in both sexes.[8,9] The cutoff recommended by WHO to be used to determine folate deficiency is < 4 ng/mL (< 10 nmol/L) in serum, the same cutoff can be applied to all ages.[6]

During pregnancy, the mother undergoes both anatomical and physiological changes to enable the fetus to develop and grow. These changes include a progressive increase in plasma volume, but the expansion of plasma volume is greater than the increase in red blood cell mass, which leads to a fall in the hemoglobin concentration, haematocrit and RBC count.[10] These changes may influence the folate concentrations in pregnant women.

Folate is essential for fetal development, and guidelines recommend women that are pregnant or are planning to become pregnant to take folic acid supplements at a concentration of 400 µg/day to prevent fetal malformations such as neural tube defects, but also other pregnancy complications such as preeclampsia.[11,12,13] If not supplemented during pregnancy and lactation, folate levels decrease in both plasma and RBC.[14] Folic acid supplements of 400 µg/day are to ensure that the women achieve an RBC folate cutoff of 906 nmol/L, which is the value associated with maximal reduction of the risk of neural tube defect.[15,16] By examining the association between the folate concentrations in plasma and in RBC, an estimated plasma-folate insufficiency cutoff of 25.5 nmol/L was found to correspond to the RBC-folate insufficiency cutoff of 906 nmol/L.[17]

A clinical manifestation of both folate and vitamin B12 deficiency is the so called megaloblastic (macrocytic) anemia: due to the affected DNA synthesis and cell maturation, especially involving the cells of erythropoiesis, the total count of erythrocytes is significantly reduced. The hemoglobin synthesis capacity however is normal, which leads to abnormally large erythrocyte precursors ("macrocytes" or "megaloblasts"), which have an elevated hemoglobin content ("hyperchromic anemia").[3,18]

Because vitamin B12 and folate are closely interrelated in the cellular one-carbon-unit metabolism, and also hematologic and clinical consequences of the two vitamin-deficiency states might be similar, it is advisable to determine both parameters simultaneously in patients with the relevant symptoms of vitamin-deficiency.[3,18]

1. Folate serum/plasma application

Test principle

Competition principle. Total duration of assay: 27 minutes.

- 1st incubation: By incubating 15 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 II 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 **cobas** link.

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as FOL 3.

PT1 Pretreatment reagent 1, 1 bottle, 7.3 mL:
Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2 Pretreatment reagent 2, 1 bottle, 7.3 mL:
Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

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R1 Folate-binding protein~Ru(bpy)$_3^{2+}$, 1 bottle, 16.7 mL:
Ruthenium-labeled folate-binding protein 75 µg/L; human serum albumin (stabilizer); phosphate buffer 70 mmol/L, pH 5.5; preservative.

R2 Folate~biotin, 1 bottle, 13.9 mL:
Biotinylated folate 17 µg/L; human serum albumin (stabilizer); bis-tris propane buffer 100 mmol/L, pH 9.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

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.

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

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

Response:

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

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

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

P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling 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 donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.

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.[19,20]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The Elecsys Folate III kit can be used for both the folate serum/plasma application and the folate RBC application.

Both applications use the same reagents.

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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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
on the analyzers	16 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: Slope 0.9-1.1, coefficient of correlation ≥ 0.95.

Stable for 2 hours at 20-25 °C, 48 hours at 2-8 °C, 28 days at -20 °C (± 5 °C). Freeze only once. 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.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

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

Due to possible evaporation effects, samples and calibrators 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 08324247190, CalSet Folate, for 4 x 1.0 mL
- REF 05618860190, PreciControl Varia, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M

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- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This application has been standardized against the WHO International Standard NIBSC Code 03/178.

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 **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack 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.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, 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.

Calculation

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

Conversion factors: nmol/L x 0.44 = ng/mL

ng/mL x 2.27 = nmol/L

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 496 µmol/L or ≤ 29 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1000 IU/mL
IgG	≤ 1.6 g/dL
IgA	≤ 0.4 g/dL
IgM	≤ 1 g/dL

Criterion: For concentrations of 0.6-4 ng/mL the deviation is ≤ 0.4 ng/mL. For concentrations > 4 ng/mL the deviation is ≤ 10 %.

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 with extremely high total protein concentrations (hyperproteinemia) are not suitable for use in this assay. Hyperproteinemia may be caused by, but not limited to, the following conditions: Lymphoma,[21,22] bone marrow disorders such as multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), Waldenström macroglobulinemia, plasmocytoma,[21,22,23,24,25,26,27] amyloidosis.[27,28] Respective samples may lead to the formation of protein gel in the assay cup, which may cause a run abort. The critical total protein concentration is dependent upon the individual sample composition.

Pharmaceutical substances

In vitro tests were performed on 15 commonly used pharmaceuticals. No interference with the assay was found. For the common pharmaceuticals cefoxitin and doxycycline no interference was observed for concentrations ≤ 250 mg/L and ≤ 6 mg/L, respectively.

In addition, the following special drug was tested. No interference with the assay was found.

Special drug

Drug	Concentration tested U/mL
Erythropoietin	2000

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.

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 the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.6-20.0 ng/mL or 1.36-45.4 nmol/L (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.6 ng/mL or < 1.36 nmol/L. Values above the measuring range are reported as > 20.0 ng/mL or > 45.4 nmol/L (or up to 40.0 ng/mL or 90.8 nmol/L for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.6 ng/mL (1.36 nmol/L)

Limit of Detection = 1.2 ng/mL (2.72 nmol/L)

Limit of Quantitation = 2.0 ng/mL (4.54 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

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The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

It has been determined using low concentration folate samples.

Dilution

Samples with folate concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:2 (either automatically by the analyzers or manually). The concentration of the diluted sample must be ≥ 8.5 ng/mL or ≥ 19.3 nmol/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

Referring to "The American Journal of Clinical Nutrition"[29] serum folate (folic acid) values were found as follows:

Sex	Age	N	Median		2.5th-97.5th percentile	
	years		ng/mL	nmol/L	ng/mL	nmol/L
Both	all	23345	13.0	29.5	4.6-34.8	10.4-78.9
Male	all	11387	12.3	27.9	4.5-32.2	10.2-73.0
Female	all	11958	13.6	30.1	4.8-37.3	10.9-84.5
Both	4-11	3595	17.2	39.0	8.6-37.7	19.5-85.4
Both	12-19	6390	12.1	27.4	5.0-27.2	11.3-61.6
Both	20-59	8689	11.6	26.3	4.4-31.0	10.0-70.2
Both	≥ 60	4671	16.6	37.6	5.6-45.8	12.7-103.8

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, REF 07559992190.

The calculation is based on 404 sera (177 men, 227 women). The age range was between 20 and 65 years. Pregnant or lactating women were excluded. The reference population was selected according to normal homocysteine values.

N	Median		2.5th-97.5th percentile	
	ng/mL	nmol/L	ng/mL	nmol/L
404	8.94	20.3	3.89-26.8	8.83-60.8

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[a)] 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.

a) Folate deficiency was assessed by measurement of serum folate by 2 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, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Human serum 1	4.40	0.336	7.6	0.390	8.9
Human serum 2	8.74	0.447	5.1	0.488	5.6
Human serum 3	10.8	0.384	3.6	0.447	4.1
Human serum 4	21.9	0.804	3.7	0.888	4.1
Human serum 5	41.1	1.31	3.2	1.38	3.4
PreciControl Varia 1	10.2	0.456	4.5	0.511	5.0
PreciControl Varia 2	28.4	0.817	2.9	1.20	4.2

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	1.94	0.148	7.6	0.172	8.9
Human serum 2	3.85	0.197	5.1	0.215	5.6
Human serum 3	4.74	0.169	3.6	0.197	4.1
Human serum 4	9.66	0.354	3.7	0.391	4.1
Human serum 5	18.1	0.576	3.2	0.607	3.4
PreciControl Varia 1	4.49	0.201	4.5	0.225	5.0
PreciControl Varia 2	12.5	0.360	2.9	0.529	4.2

Method comparison

a) A comparison of the Elecsys Folate III serum/plasma application, REF 08324174190 (**cobas e** 801 analyzer; y), with the Elecsys Folate III serum/plasma application, REF 07027290190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 142

Passing/Bablok[30]	Linear regression
y = 1.08x + 0.136	y = 1.07x + 0.161
τ = 0.942	r = 0.997

The sample concentrations were between 0.621 and 19.1 ng/mL (1.41 and 43.4 nmol/L).

b) A comparison of the Elecsys Folate III serum/plasma application, REF 08324174190 (**cobas e** 402 analyzer; y), with the Elecsys Folate III serum/plasma application, REF 08324174190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 143

Passing/Bablok[30]	Linear regression
y = 0.968x + 0.264	y = 0.961x + 0.322
τ = 0.913	r = 0.995

The sample concentrations were between 1.69 and 19.9 ng/mL (3.84 and 45.2 nmol/L).

Analytical specificity

The following cross-reactivities were found, tested with a folate concentration of approximately 4 ng/mL.

Cross-reactant	**Concentration tested ng/mL**	**Cross-reactivity %**
Amethopterin	750	0.6
Aminopterin	750	1.7
Folinic acid	750	0.5

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2. Folate RBC application

Test principle

Competition principle. Total duration of assay: 27 minutes.

Whole blood treated with anticoagulants (heparin or EDTA) is mixed with ascorbic acid solution and incubated for approximately 90 minutes at 20-25 °C. Lysis of the erythrocytes takes place, with liberation and stabilization of the intracellular folate. The resulting hemolysate sample is then used for subsequent measurement.

- 1st incubation: By incubating 15 µL of hemolysate 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 II 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 **cobas** link.

Reagents - working solutions

The **cobas e** pack (M, R1, R2) and the pretreatment reagents (PT1, PT2) are labeled as FOL 3.

PT1 Pretreatment reagent 1, 1 bottle, 7.3 mL:
Sodium 2-mercaptoethanesulfonate (MESNA) 40 g/L, pH 5.5.

PT2 Pretreatment reagent 2, 1 bottle, 7.3 mL:
Sodium hydroxide 25 g/L.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Folate-binding protein~Ru(bpy)$_3^{2+}$, 1 bottle, 16.7 mL:
Ruthenium-labeled folate-binding protein 75 µg/L; human serum albumin (stabilizer); phosphate buffer 70 mmol/L, pH 5.5; preservative.

R2 Folate~biotin, 1 bottle, 13.9 mL:
Biotinylated folate 17 µg/L; human serum albumin (stabilizer); bis-tris propane buffer 100 mmol/L, pH 9.0; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
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This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

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Prevention:

P261 Avoid breathing mist or vapours.

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

Response:

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

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Reagent handling

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Both applications use the same reagents.

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Storage and stability

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Do not freeze.

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Stability:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Hemolysate prepared from whole blood treated with anticoagulants (Na-heparin or K_3-EDTA).

- *For the determination of folate in RBC*
  Determine hematocrit in whole blood samples and record the value.

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- *Preparation of the hemolysate sample*
  Mix 3.0 mL of Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %) and 100 µL of well-mixed whole blood, avoiding foam formation. Incubate with closed caps for 90 ± 15 minutes at 20-25 °C.

Stability:
If the hemolysate sample is prepared from fresh whole blood, it is possible to store the prepared hemolysate sample for 28 days at -20 °C (± 5 °C). Freeze only once. Analyze the sample promptly after thawing.

Whole blood storage prior to hemolysate preparation: 2 hours at 20-25 °C,[31] 24 hours at 2-8 °C, 28 days at -20 °C (± 5 °C; only EDTA blood). Freeze only once. If the whole blood sample was stored in one of these ways, the hemolysate sample must be used directly after preparation.

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.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

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

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

If measurements cannot be carried out within 2 hours please store the hemolysate sample at -20 °C (± 5 °C).

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 08324247190, CalSet Folate, for 4 x 1.0 mL
- REF 05944317190, Folate RBC Hemolyzing Reagent kit for 4 x 200 mL, contains ascorbic acid
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

The well-mixed hemolysate sample is placed in the sample zone of the analyzer and recorded by entering the sample identification data. Complete determinations on the analyzer within 2 hours after finalizing the preparation of the hemolysate sample.

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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibration

Traceability: This application has been standardized against the Elecsys Folate III assay (REF 04476433190)/RBC application.

The standardization of the folate RBC application includes the volume correction to account for the preparation of hemolysate sample (1:31 vol/vol).

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 **cobas e** pack was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use commercially available whole blood control material.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per **cobas e** pack, 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.

Calculation

1. Whole blood folate (from hemolysate sample)

The standardization of the folate RBC application includes the volume correction to account for the preparation of hemolysate sample (1:31 vol/vol).

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

Conversion factors: nmol/L x 0.44 = ng/mL

ng/mL x 2.27 = nmol/L

2. RBC folate

To calculate the folate concentration in the erythrocyte fraction of the sample **(RBC folate)**, the predetermined sample specific hematocrit value must be taken into account using the following equation:

$$\text{RBC folate} = \frac{\text{analyzer result}}{\text{\% hematocrit}} \times 100$$

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 496 µmol/L or ≤ 29 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1000 IU/mL
IgG	≤ 1.6 g/dL
IgA	≤ 0.4 g/dL
IgM	≤ 1 g/dL

Criterion: For concentrations of 120-210 ng/mL the deviation is ≤ 21 ng/mL. For concentrations > 210 ng/mL the deviation is ≤ 10 %.

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Pharmaceutical substances
In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drug was tested. No interference with the assay was found.

Special drug

Drug	Concentration tested U/mL
Erythropoietin	2000

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.

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

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

In rare cases, samples with low erythrocyte folate concentration, but high serum folate concentration can occur. In these cases, a correction of the folate concentration in erythrocytes by the serum folate concentration with the following equation is recommended*:

* expected values can be used as an indicator for high serum folate concentration

Corrected RBC folate concentration =

$$\text{RBC folate concentration} - \left(\text{serum folate concentration} \times \frac{100 - \%\ \text{hematocrit}}{\%\ \text{hematocrit}}\right)$$

Example

RBC folate concentration: 241 (ng/mL RBC);

serum folate concentration: 10.5 (ng/mL S);

hematocrit measured (%) = 45

Corrected RBC folate concentration =

$$241\ \text{ng/mL RBC} - \left(10.5\ \text{ng/mL S} \times \frac{100 - 45}{45}\right) = 228\ \text{ng/mL RBC}$$

Limits and ranges

Measuring range

120-620 ng/mL or 272-1407 nmol/L (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 120 ng/mL (< 272 nmol/L). Values above the measuring range are reported as > 620 ng/mL (> 1407 nmol/L). Values are not corrected for the sample hematocrit.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation:

Limit of Blank = 45 ng/mL (102 nmol/L)

Limit of Detection = 70 ng/mL (159 nmol/L)

Limit of Quantitation = 120 ng/mL (272 nmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 30 %.

It has been determined using low concentration folate samples.

Dilution

Hemolysate samples with folate concentrations above the measuring range can be diluted manually with Elecsys Folate RBC Hemolyzing Reagent (ascorbic acid solution, 0.2 %). The recommended dilution is 1:2. The concentration of the diluted sample must be ≥ 265 ng/mL or ≥ 602 nmol/L. After manual dilution, multiply the results by the dilution factor 2.

Expected values

The values shown below were measured on samples from an apparently healthy population, using the Elecsys Folate III/RBC application. The values can be applied for the folate RBC application on all Elecsys and **cobas e** analyzers. The calculation is based on 290 sera (96 men, 194 women) from an European population. 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. The following values were obtained:

Whole blood folate (from hemolysate samples)					
	N	Median		2.5th-97.5th percentile	
		nmol/L	ng/mL	nmol/L	ng/mL
Europe	290	673	296	481-1212	212-534

The measured hematocrit value in this study showed a range from 37.1-46.1 %.

RBC folate (folate in erythrocyte fraction)					
	N	Median		2.5th-97.5th percentile	
		nmol/L	ng/mL	nmol/L	ng/mL
Europe	290	1657	730	1187-2854	523-1257

If pathologically low hematocrit values are considered for calculation of RBC folate in the erythrocyte fraction, elevated RBC folate concentrations may be observed. No medical conclusion should be based on the calculation considering hematocrit values in such cases. Instead, whole blood folate results (from hemolysate samples) and suitable expected values may be used.

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

Precision was determined using Elecsys reagents and hemolysate samples in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). Results are given as whole blood folate (from hemolysate sample). The following results were obtained:

cobas e 801 and **cobas e** 402 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean nmol/L	SD nmol/L	CV %	SD nmol/L	CV %
Hemolysate 1	345	13.0	3.8	14.0	4.1
Hemolysate 2	468	13.9	3.0	16.3	3.5
Hemolysate 3	572	15.2	2.7	18.8	3.3
Hemolysate 4	824	18.2	2.2	22.7	2.8
Hemolysate 5	1373	24.3	1.8	33.1	2.4

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cobas e 801 and **cobas e** 402 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Hemolysate 1	152	5.73	3.8	6.17	4.1
Hemolysate 2	206	6.14	3.0	7.17	3.5
Hemolysate 3	252	6.70	2.7	8.28	3.3
Hemolysate 4	363	8.01	2.2	10.0	2.8
Hemolysate 5	605	10.7	1.8	14.6	2.4

Method comparison

a) A comparison of the Elecsys Folate III RBC application, REF 08324174190 (**cobas e** 801 analyzer; y), with the Elecsys Folate III RBC application, REF 07027290190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 123

Passing/Bablok[30]	Linear regression
y = 1.04x - 12.3	y = 1.02x - 8.91
τ = 0.916	r = 0.992

The sample concentrations were between 132 and 618 ng/mL (300 and 1403 nmol/L).

b) A comparison of the Elecsys Folate III RBC application, REF 08324174190 (**cobas e** 402 analyzer; y), with the Elecsys Folate III RBC application, REF 08324174190 (**cobas e** 801 analyzer; x), using clinical samples gave the following correlations (ng/mL):

Number of samples measured: 142

Passing/Bablok[30]	Linear regression
y = 0.950x - 8.33	y = 0.947x - 8.34
τ = 0.923	r = 0.994

The sample concentrations were between 128 and 617 ng/mL (291 and 1401 nmol/L).

Analytical specificity

The following cross-reactivities were found, tested with a folate concentration of approximately 210 ng/mL.

Cross-reactant	**Concentration tested ng/mL**	**Cross-reactivity %**
Amethopterin	750	1.7
Aminopterin	750	2.0
Folinic acid	750	2.6

References

1 Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene 2014 Jan 1;533(1):11-20. doi: 10.1016/j.gene.2013.09.063.

2 Scaglione F, Panzavolta G. Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. Xenobiotica 2014 May;44(5):480-488. doi: 10.3109/00498254.2013.845705.

3 Reynolds EH. The neurology of folic acid deficiency. Handb Clin Neurol 2014;120:927-943. doi: 10.1016/B978-0-7020-4087-0.00061-9.

4 Bronsky J, Campoy C, Braegger C; ESPGHAN/ESPEN/ESPR/CSPEN working group on pediatric parenteral nutrition. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Vitamins. Clin Nutr 2018 Dec;37(6 Pt B):2366-2378. doi: 10.1016/j.clnu.2018.06.951.

5 Glader B. Anemias of inadequate production. In: Kliegman, Robert M.; Stanton, Bonita M.D.; Jenson, Hal B.; Behrman, Richard E, editors. Nelson textbook of pediatrics. 18th ed. Philadelphia: Saunders Elsevier; 2007. p. 2006-2018

6 WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2012 (https://apps.who.int/iris/bitstream/handle/10665/75584/WHO_NMH_NHD_EPG_12.1_eng.pdf?sequence=1)

7 Snow CF. Laboratory Diagnosis of Vitamin B12 and Folate Deficiency: A Guide for the Primary Care Physician. Archives of Internal Medicine 1999;159(12):1289-1298.

8 Monsen AL, Refsum H, Markestad T, et al. Cobalamin status and its biochemical markers methylmalonic acid and homocysteine in different age groups from 4 days to 19 years. Clin Chem 2003 Dec;49(12):2067-2075. doi: 10.1373/clinchem.2003.019869.

9 Kreusler P, Vogel M, Willenberg A, et al. Folate and Cobalamin Serum Levels in Healthy Children and Adolescents and Their Association with Age, Sex, BMI and Socioeconomic Status. Nutrients 2021 Feb 7;13(2):546. doi: 10.3390/nu13020546.

10 Soma-Pillay P, Nelson-Piercy C, Tolppanen H, et al. Physiological changes in pregnancy. Cardiovasc J Afr 2016 Mar-Apr;27(2):89-94. doi: 10.5830/CVJA-2016-021.

11 WHO antenatal care recommendations for a positive pregnancy experience. Nutritional interventions update: Multiple micronutrient supplements during pregnancy. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

12 Tuncalp Ö, Rogers LM, Lawrie TA, et al. WHO recommendations on antenatal nutrition: an update on multiple micronutrient supplements. BMJ Glob Health 2020 Jul;5(7):e003375. doi: 10.1136/bmjgh-2020-003375.

13 Yuan X, Han X, Zhou W, et al. Association of folate and vitamin B12 imbalance with adverse pregnancy outcomes among 11,549 pregnant women: An observational cohort study. Front Nutr 2022 Jul 25;9:947118. doi: 10.3389/fnut.2022.947118.

14 Milman N, Byg KE, Hvas AM, et al. Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women. Eur J Haematol 2006 Mar;76(3):200-5. doi: 10.1111/j.1600-0609.2005.00606.x.

15 Patti MA, Braun JM, Arbuckle TE, et al. Associations between folic acid supplement use and folate status biomarkers in the first and third trimesters of pregnancy in the Maternal-Infant Research on Environmental Chemicals (MIREC) Pregnancy Cohort Study. Am J Clin Nutr. 2022 Dec 19;116(6):1852-1863. doi: 10.1093/ajcn/nqac235.

16 Daly LE, Kirke PN, Molloy A, et al. Folate levels and neural tube defects. Implications for prevention. JAMA 1995 Dec 6;274(21):1698-1702. doi: 10.1001/jama.1995.03530210052030.

17 Chen MY, Rose CE, Qi YP, et al. Defining the plasma folate concentration associated with the red blood cell folate concentration threshold for optimal neural tube defects prevention: a population-based, randomized trial of folic acid supplementation. Am J Clin Nutr. 2019 May 1;109(5):1452-1461. doi: 10.1093/ajcn/nqz027.

18 Wick M, Pinggera W, Lehmann P. Clinical Aspects and Laboratory. Iron metabolism, Anemias. Springer Verlag, Wien, New York, 6th edition 2011:41-42.

19 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

20 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.

21 Wu AHB. Tietz clinical guide to laboratory tests, 4th ed. St. Louis, Saunders/Elsevier 2006:608-609, 916-917.

22 Paricaud K, Moulis G, Combis MS, et al. Causes of protidemia above 100 g/L. Eur J Intern Med 2014;25:e123.

23 Filippatos TD, Liamis G, Christopoulou F, et al. Ten common pitfalls in the evaluation of patients with hyponatremia. Eur J Intern Med 2016;29:22-25.

24 Mailankody S, Landgren O. Monoclonal gammopathy of undetermined significance and Waldenström's macroglobulinemia. Best Pract Res Clin Haematol 2016;29:187-193.

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25 Morel P, Duhamel A, Gobbi P, et al. International prognostic scoring system for Waldenström macroglobulinemia. Blood 2009;113:4163-4170.

26 Rajkumar SV. Multiple Myeloma. Curr Probl Cancer 2009;33:7-64.

27 Gertz MA. Immunoglobulin light chain amyloidosis: 2016 update on diagnosis, prognosis, and treatment. Am J Hematol 2016;91:947-956.

28 Wu AHB. Tietz clinical guide to laboratory tests, 4th ed. St. Louis, Saunders/Elsevier 2006: 916-917, 925.

29 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.

30 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

31 Eijsden M, van der Wal MF, Hornstra G, et al. Can whole blood samples be stored over 24 hours without compromising stability of C-Reactive Protein, Retinol, Ferritin, Folic Acid and Fatty Acids in Epidemiology Research? Clin Chem 2005;51(1):230-232.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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.

Any serious incident that has 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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche