Instruction For Use

【Product Name】
Folate (Electrochemiluminescence Immunoassay)

【Package】

REF NO.	Package Size
694020	2×50T
694019	50T
694018	100T
694021	2×100T

【Intended Use】
Immunoassay for the in vitro quantitative determination of folate in human serum.

【Summary】
Folate is a kind of vitamin compound with similar chemical structure and biological activity. It belongs to B vitamins, also known as vitamin B11 or vitamin Bc. Folate is a combination of acridinium, p-aminobenzoic acid and L-glutamic acid. If glutamic acid is removed, vitamin activity is lost. Folate, as a vitamin, is a kind of organic substance necessary for maintaining the normal life process of the body. Although it is rarely needed, it is very important for maintaining health and is necessary for normal metabolism, nucleic acid synthesis, amino acid metabolism and erythropoiesis. In different metabolic pathways, different metabolically active forms of folate can be formed due to the action of different enzymes, wherein 5-methyltetrahydrofolate is the main form of the folate cycle. Folate and vitamin B12 are essential nutrients for human hematopoiesis. Giant erythrocyte anemia is almost always caused by the lack of one of these two vitamins. Folate deficiency can cause trophic and megaloblastic anemia, poor physiological function, gastrointestinal dysfunction, mental deterioration, and the foetus neural tube defect[1]. The cause of folate deficiency may be due to the following reasons: inadequate dietary intake (lack of natural fruits, vegetables or other foods containing folic acid), malabsorption caused by gastrointestinal diseases, chronic alcoholism, addictive drugs (oral contraceptives, folate antagonists) treatment, pregnancy (increased demand for folate), and the elderly or low-economic population, etc. Lack of diet and malabsorption are the most important causes of folate deficiency in humans[2-4].

【Test Principle】
Competition principle. Total duration of assay: 27 minutes
- 1st incubation: By incubating 25 µL of the sample with pretreatment reagent II and III, bound folate is released from endogenous folate binding protein.
- 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 Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is immediately measured by a photomultiplier.
- Results are determined via a calibration curve which is specifically generated by 2 point calibration and a master curve provided via the reagent barcode.

【Main Component】
The reagent pack consists of MB, RA, RB, PT2, PT3, calibrators and control materials (Optional). Different lots cannot be used at the same time or mixed up together.

Components	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
Pretreatment reagent II (PT2)	Sodium 2-mercaptoethanesulfonate (MESNA)	1×0.8 mL	2×0.8 mL	1×1.6 mL	2×1.6 mL
Pretreatment reagent III (PT3)	Sodium Hydroxide	1×0.6 mL	2×0.6 mL	1×1.1 mL	2×1.1 mL
MB	Streptavidin-coated microparticles (0.75mg/mL), preservative	1×1.5 mL	2×1.5 mL	1×3.0 mL	2×3.0 mL
RB	Biotin labeled folate coupled BSA, HEPES, preservative	1×2.0 mL	2×2.0 mL	1×4.0 mL	2×4.0 mL
RA	Folate binding protein ~$Ru(bpy)_3^{2+}$, HEPES, preservative	1×3.0 mL	2×3.0 mL	1×6.0 mL	2×6.0 mL
Calibrators	Horse serum, preservative, lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			
Control Materials (Optional)	Horse serum, preservative, lyophilized	High: 1×1.0 mL Low: 1×1.0 mL			

Traceability: This kit can be traced back to the folate international standard materials (NIBSC code: 03/178).
See the control material value sheet for the target values and ranges of control materials.
Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)
Additional materials for Automated ECL Immunoassay Analyzer eCL8000, eCL8000i, eCL8000p, eCL8000x:
- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:
- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:
- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Storage and Shelf Life】
Store at 2ºC-8°C.
Do not freeze.
Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
Stored on the analyzers at 2ºC~15ºC	8 weeks

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

Stability of the lyophilized calibrators and control materials	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after reconstituted	3 days
Store at -25ºC~-15ºC after reconstituted	3 months (freeze only once, Saved away from light.)

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

【Specimen collection, handling and storage】
Serum collected using standard sampling tubes or tubes containing separating gel. Blood samples should be collected by standard operation of venous puncture; After complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not recommended. 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. Sample for folate determinations should be collected from fasting persons. Samples should be tested in 2 hours after collection, otherwise, stored at 2~8ºC for no more than 2 days or -20ºC for 1 months. Freezing and thawing cycle is permitted only once. Saved away from light. If the sample is not tested in time, please save it at 2~8 °C.

【Assay Procedure】
Testing procedures and precautions
Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.
Set up Folate test according to the operational manual.
Put the Folate rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 minutes before testing.
Recommended ambient temperature:10~30°C; Relative humidity: ≤ 80%.
Handing of lyophilized calibrators and control materials:
Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 10~15 minutes to reconstitute. Mix carefully, avoiding foam formation. Transfer aliquots of the reconstituted calibrators or control materials to the additional bottles. After bottles is marked and dispensed. If you do not test immediately, you need to store the aliquots immediately at -20 ℃ (± 5 ℃).
Calibration
Calibration should be performed using lot-matching reagent and calibrators.
Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (Primary and Secondary RFID) reagent card (refer to instrument user manual). The main curve is adjusted into specific working curve according to the calibrator results, and thereafter the

judge whether the working curve is qualified or not.
Recalibration is recommended when:
(1) the reagents of different lot are used;
(2) the same lot of reagents were used on the analyzer beyond 4 weeks;
(3) Quality control findings outside the defined limits.
(4)The Buffer of different lots are used.

Quality control

For quality control, use Folate control materials.
In addition, other suitable control material can be used.
In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. After each calibration, reagent lot change, maintenance or failure repairment, quality control is recommended. The quality control results should fluctuate within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be rechecked.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL or ng/mL: ng/mL × 2.27 = nmol/L

Specimen dilution

Samples containning the testing targeted materials with concentration beyond the measuring range, must be manually diluted with folate -negative serum.
The recommended dilution ratio is 1:2(V_{sample}:$V_{folate\text{-}negative\ serum}$), the concentration of the diluted sample must be > 8.5 ng/mL, and the diluted sample test results need to multiply dilution ratio.

【Biological reference interval】

According to serum test results of 240 healthy people (including 120 males and 120 females) in Guangdong Province, P. R. China (excluding pregnant and lactating women), the percentile 2.5 to 97.5 gives reference range : 3.6-19.6 ng/mL.Due to differences in the region, race, gender and age, laboratories are recommended to set up their own reference ranges if necessary.

【Result Interpretation】

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

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.
When the sample containing bilirubin ≤564 μmol/L or 33 mg/dL, triglyceride ≤1500 mg/dL, biotin ≤86.1 nmol/L or 21 ng/mL, RF (rheumatoid factors) ≤1000IU/Ml, or HAMA (human anti-murine antibodies) ≤ 759.78 ng/mL, interference deviation to the test result lies within ±10%.

【Measuring Range】

The measuring range of the kit is 0.60~20.0 ng/mL. For samples with folate content below the lower detection limit, the results are reported as < 0.6 ng/mL; If the folate content is above the upper detection limit, the results are reported as >20.0 ng/mL.

【Analytical Performance】

Lower limit of measurement

The lower detection limit is 0.60 ng/mL (repeatability study, n = 20).

Accuracy

The ratio of measuring international standard materials between the measured value and the theoretical value should be between 0.900 and 1.100.

Linearity

The correlation coefficient (Pearson's r) is not less than 0.9900 in the interval of 0.60~20.0 ng/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than 6%.

Between-lot precision

The coefficient of variation (CV) is less than 10%.

Analytical specificity

Test results of blank samples spiked with analogs below are summarized in the following table.

Analog	Concentration	Cross-reactivity (%)
Aminopterin	750 ng/mL	≤1.0
Folinic acid	750 ng/mL	≤1.0
Methotrexate	750 ng/mL	≤1.0

Homogeneity of Calibrators and Control Materials

Within-bottle homogeneity

The coefficient of variation (CV) is less than 10%.

Between-bottle homegeneity

The coefficient of variation (CV) is less than 10%.

Method comparison

A comparison of the Lifotronic Folate assay (y) with the Roche Elecsys Folate method (x) using clinical samples gave the following correlations:
Number of samples measured: 223
Linear regression:
y=0.9809x-0.1511
r = 0.9884
The sample concentrations were between approx 0.876 and 18.79 ng/mL.

【Precaution and Warning】

The kit is only used for in vitro diagnostics.
When using the kit, it's necessary to exercise the normal precautions in the laboratory.
All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.

Test results of the kit and the kit clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.
Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest lead to the wrong medical explanation; It's suggested that laboratorians should provide the characteristics of used reagent in the test report to the clinician. In serial monitoring, continuous parallel tests should be performed and the results must be compared with the former to determine a new baseline value if the reagent type is changed.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant. The abandoned reagents should be disposed with in compliance with local regulations.

【Symbol Explanation】

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

【Reference】

1. Lindenbaum J. Status of laboratory testing in the diagnosis of megaloblastic anemia. Blood 1983; 61:627-7.
2. Herbert V. The nutritional anemias. Hosp Pract 1980 ;15(3):65-83,87-9.
3. Billen J., Zaman Z., Claeys G. et al. Limited Dynamic range of a new assay for serum folate, Clin Chem 1999; 45(4):581-2.
4. Klee GC. Cobalamin and folate evaluation: measurement of methylmalonic acid and homocysteine vs vitamin B12 and folate. Clin Chem 46; 2000:1277-1283.

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