MAGLUMI FA (CLIA)

REF 130213001M Σ 100

EC REP

Shenzhen New Industries Biomedical Engineering Co., Ltd
4F,Wearnes Tech Bldg,
Science & Industry Park,
Nanshan,Shenzhen,518057CHINA
Tel. + 86-755-86028224
Fax.+ 86-755-26654850

Lotus Global Co., Ltd
15 Alexandra Road
London
NW8 0DP
UK
Tel. + 44-20-75868010
Fax.+ 44-20-79006187

CE

FOR PROFESSIONAL USE ONLY
Store at 2...8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

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SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in Europe
	Manufacturer
	Attention. See Instructions For Use
CONT	Contents of kit
IVD	In vitro diagnostic medical device (In vitro diagnostic use)
LOT	Lot number
REF	Catalogue Code
	Expiry date (Use by…)
2°C 8°C	Temperature limitation (store at 2...8 °C)
Σ	Number of tests
	Keep away from direct sunlight
	Keep upright for storage

INTENDED USE

The kit has been designed for the quantitative determination of Folic Acid (FA) in human serum.
The method can be used for samples over the range of 0-24 ng/ml. The test has to be performed on the MAGLUMI chemiluminescence immunoassay (CLIA) fully auto analyzer (Including MAGLUMI 1000, MAGLUMI 2000, MAGLUMI 2000 Plus and new developed models).

SUMMARY AND EXPLANATION OF THE TEST

Folates do play an important role in the synthesis of nucleic acids and some amino acids and gained recently due to their belonging to the group of antioxidative working Vitamins increased interest. In the last years increasingly the influence of FA supplementation to avoid abortion and dysraphism was a topic of research. Folic acid as most stable representative of the group of Folates is added to a broad range of food.
Traditional methods are mostly microbiological methods, but also TLC and HPLC, where all the described methods are linked to a big time consumption and need a lot of equipment.
This test kit allows the quicker detection (2.5 to 4 hours. including sample preparation) of folic acid in supplemented food compared to traditional techniques (24-48 hrs).

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay;
Use a purified FA antigen to label ABEI, and use an FA-binding Protein to label FITC. Sample, Calibrator or Control with ABEI label, FITC label and magnetic microbeads are mixed thoroughly and incubated at 37℃, forming antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of FA present in controls or samples.

CONT KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer,1.2% (W/V), 0.2%NaN_3, coated with sheep anti-FITC polyclonal antibody..	2.5ml
Calibrator Low: bovine serum, 0.2%NaN_3.	3.0ml
Calibrator High: bovine serum, 0.2%NaN_3	3.0ml
Displacing Reagent (preparation required)	
FITC Label: FA-binding Protein labeled FITC, containing BSA, 0.2%NaN_3.	12.5ml
ABEI Label: purified FA antigen labeled ABEI, containing BSA, 0.2%NaN_3.	7.5ml
Prepare Displacing Reagent before using the integral	

Reagent Vials in kit box	
Empty bottle of displacing reagent	6 bottles
NaOH Solution: 0.5M NaOH	15ml
DTT: lyophilized 30mg DTT, reconstituted with 300µl distilled water	300µl
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation!) Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Preparation of Displacing Reagent

1. Use 300µl distilled water to dissolve the lyophilized DTT completely in DTT reagent vial, then aliquot into 3 empty tubes by 100µl each (Eppendof tube, 0.5ml type is recommended). The dissolved DTT tube should be sealed and stored at -20℃ and can be stable for 2 month. Take it out to room temperature before preparing Displacing Reagent.
2. Pipette 5ml NaOH into the empty displacing reagent bottle, then piptette 50µl DTT solution from DTT tube and add in the displacing reagent bottle. Using the NaOH solution in the bottle to rinse the wall, make sure DTT solution is dissolved with NaOH solution completely. Then horizontally shake the bottle in round cycles for mixing, avoided forming bubbles.
3. Place Displacing Reagent bottle into the 4th position of the kit integral. This bottle can be used for about 100 tests. (It is recommended customer collect enough samples and run the tests together).

Note:

- The prepared displacing reagent can only be used for 108 hours at 2-8℃. After 108 hours, the displacing reagent will be expired, and it should be discarded
- Displacing reagent shelf life will be shorted if place on the analyzer, it is recommended to finish the displacing reagent in 48 hours to ensure its performance.

Storage and Stability

- Sealed: Stored at 2-8 ℃ until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.
- Keep upright for storage.
- Keep away from direct sunlight.

CALIBRATION AND TRACEABILITY

1)Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the WHO International Standard Folate, Whole Blood Haemolysate 95/528.

2) 2-Point Recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

3) Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the MAGLUMI Fully Auto analyzer.
- If controls are beyond the expected range.

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect samples using standard procedures.

Store at 2-8 ℃: 24 hours, for lo

below - 20 ℃

Avoid repeated freezing and thawing cycles, stored samples should be thoroughly mixed prior to use (Vortex mixer).

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.
(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- The sample which has been placed **at the room temperature more than 8 hours cannot be used again**.
- The sample serum with **high concentration of protein (>160g/L) cannot be used** to do the tests. As the high dose of protein will form the gel and block the needle
- The Folic Acid is sensitive with light, please **avoid exposure to sunlight** when collect specimen
- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens and controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum or plasma from the serum or plasma separator, red blood cells or clot. Specimens removed from the separator gel, cells or

clot may be stored up to 24 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping
- Before shipping specimens, it is recommended that specimens be removed from the serum or plasma separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.
- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 2000, p.2143). A minimum of one hour at 121 ℃ is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions
- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the MAGLUMI Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the MAGLUMI Chemiluminescence Analyzer Operating Instructions.

100μl +40μl	Sample , Calibrator Displacing reagent
2min	incubation
+50μl +100μl +20μl	ABEI label FITC label Nano magnetic microbeads
15 min	Incubation
400μl	Cycle washing
3 s	Measurement

DILUTION

Sample dilution by analyzer is not available in this reagent kit
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor.
Please choose applicable diluents or ask SNIBE for advice before manual dilution must be processed.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results.
Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<0.06mg/ml, haemoglobin<16mg/dl or triglycerides< 12.5mg/ml.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

1) Calculation of Results
- The analyzer automatically calculates the FA concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/ml. For further information please refer to the MAGLUMI Chemiluminescence Analyzer

Operating Instructions.

- Conversion factor: 1 ng/ml = 2.265 nmol/L

2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were：5.21-20 ng/ml
- If the sample's value is 3.21-5.21 ng/ml, it should be checked with the clinical diagnosis and observed continuously to identify whether the patient is FA- deficiency
- Results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	8.05	0.30	3.77%
Level 2	13.72	0.42	3.06%
Level 3	19.16	0.78	4.09%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(ng/ml)	SD(ng/ml)	CV%
Level 1	8.33	0.71	8.56%
Level 2	13.81	1.12	8.09%
Level 3	20.05	1.81	9.05%

2) Analytical Sensitivity

The sensitivity is defined as the concentration of FA equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 0.375 ng/ml.

3) Specificity

The specificity of the FA assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
VB_{12}	1000 pg/ml	0.5 %

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% -110%.

Expected	Mean Measuring	Recovery
7.984 ng/ml	8.102 ng/ml	102%

5) Linearity

Use FA calibrator to prepare the six point standard curve, measuring all points' RLU except point A, and then do four parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9800.

Calibrator Point	Concentration ng/ml	Absolute linear correlation coefficient (r)
A	0	
B	1.5	r=0.9920
C	3.0	
D	6.0	
E	12.0	
F	24.0	

6) Method comparison

A comparison of MAGLUMI FA (y) with a commercially available FA test (x) using clinical samples gave the following correlations (ng/ml):

Linear regression
y = 0.92x+2.23
r = 0.974
Sy.x = 4.42

Number of samples measured: 178
The sample concentrations were between 1.2 and 21.6 ng/ml

REFERENCES

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