MAGLUMI FK506 (CLIA)

REF 130207003M

Σ 100

EC REP

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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…)
8°C 2°C	Temperature limitation (store at 2-8°C)
Σ	Number of tests
	Keep away from sunlight
	Keep upright

INTENDED USE

The kit has been designed for the quantitative determination of FK506 in human whole blood.
The method can be used for samples over the range of 0-50ng/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

FK506(also named as Tacrolimus) is an immunosuppressive drug discovered in 1984 by the Fujisawa Pharmaceutical Co.,Ltd. It has been shown to be effective for the treatment of rejection following transplantation. The results of clinical trials with liver and kidney, have been published. Clinical studies are continuing for a variety of indications.
The mode of action for FK506 is under active investigation. FK506 binds to a family of proteins termed FK506 binding proteins (FKBP's). The formation of a larger pentameric complex comprised of FKBP, FK506, calmodulin and calcineurins A and B results in the inhibition of the phosphatase activity of calcineurin. The action of transcription factors requiring dephosphorylation for transport to the cell nucleus are thus inhibited leading to blockage of T-cell proliferation and function.
FK506 may be administered IV or orally. Absorption from gastrointestinal tract is variable and irregular. Pharmacokinetic studies with FK506 have shown that there are large inter- and intra- individual differences in its kinetics in organ transplant patients.
Pharmacokinetic studies have also indicated that whole blood rather than plasma may serve as the more appropriate medium to describe the pharmacokinetic characteristics of FK506. FK506 is bound to proteins, mainly albumin, and alpha-1-acid glycoprotein, and is highly bound to erythrocytes. The distribution of FK506 between whole blood and plasma depends on several factors such as hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration. In a U.S. study, the ratio of whole blood concentration to plasma concentration ranged from 12-67 (mean 35).
FK506 is extensively metabolized in the liver and small intestine microsomes utilizing the cytochrome P-450 enzymes. Nine different metabolites of FK506 have been identified; several of the metabolites have been found and tested in whole blood.
The use of FK506 is associated with serious toxic side effects, primarily nephrotoxicity. At the present time it is not clear whether the nephrotoxicity of FK506 is the result of parent drug, metabolites, or a combination of both. Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea.

PRINCIPLE OF THE TEST

Competitive immunoluminometric assay;
Use anti-FK506 monoclonal antibody to label FITC, and purified FK506 antigen to label ABEI. Sample, Calibrator or Control, with FITC label, and magnetic microbeads are mixed thoroughly and incubated at 37 ℃, then add the antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are injected and a flash chemiluminescence reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of FK 506 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 (Lyophilized): bovine serum, 0.2%NaN_3.	2.5ml
Calibrator High (Lyophilized): bovine serum, 0.2%NaN_3.	2.5ml
FITC Label: anti-FK506 monoclonal antibody labeled FITC, containing BSA, 0.2%NaN_3.	6.5ml
ABEI Label: purified FK506 antigen labeled ABEI, containing BSA, 0.2%NaN_3.	6.5ml
Displacing reagent	4.5ml
Treat Buffer	12.5ml
Except Calibrators, other reagents are provided ready-to-use.	

Please check the chapter: SPECIMEN COLLECTION AND PREPARATION carefully for the preparation of sample.

Reagent Vials in kit box	
Red Blood Cell Lysate: 0.3M EDTA, 8.3% NH_4Cl	10.0ml
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!

Storage and Stability

- Sealed: Stored at 2-8 °C 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.

Preparation of calibrators: Dissolve the Lyophilized calibrators with distilled water according to the volume written on the tag. Shake and mix thoroughly and place it into corresponding hole of the reagent kit. (Notice: The low calibrator is beside the position of magnetic micro bead)

Storage of calibrators: Re-dissolved calibrators should be stored at -20 °C avoiding freezing and thawing more than 10 times.

CALIBRATION AND TRACEABILITY

1) Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance.

Calibrators in the reagent kit are from Sigma.

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: whole blood
- Collect 5ml of venous blood in a blank tube without additives. Add 50µl Red Blood Cell Lysate to the tube. (If you collect 2ml of venous blood in a tube, then add 20µl Red Blood Cell Lysate to the tube, following the ratio). After that, the sample can be test in the analyzer directly.
- The whole blood sample are stable for up to 12 hours at 2-8 °C. For longer storage, aliquot and store at -20 °C (up to 30 days). Avoid repeated freezing and thawing.
- If the sample is stored for a long time, mix it to make sure that the whole blood sample is uniform before testing in the analyzer

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.

(b) If use EDTA (EDTA-2K, EDTA-4Na) tube to collect sample, there is no need to add Anticoagulant reagent again.

(c) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- 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 or 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

IVD

- 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 Pathogens 13. 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.

Auto dilution +100ul +100ul	 Whole blood Treat buffer
+40ul +40ul +20ul +20ul	Auto-dil whole blood FITC label Displacing reagent Microbeads
15mins	incubation
+40µl	ABEI label
15 min	Incubation
400µl	Cycle washing
3 s	Measurement

DILUTION

Samples with concentrations above the measuring range can be diluted. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

Availability of sample dilution by analyzer please refers to the MAGLUMI analyzer user software program. Dilution settings please follow MALGUMI analyzer operating instructions.

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-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

1) Calculation of Results

- The analyzer automatically calculates the FK506 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.

2) Interpretation of Results

- Results of study in clinical centers with group of individuals, 95% of the results were 3.0-39.4ng/ml.
- 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	12.2	0.62	5.06%
Level 2	30.43	1.44	4.74%
Level 3	86.75	3.49	4.02%

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	2.33	0.19	8.04%
Level 2	31.15	2.44	7.83%
Level 3	89.23	7.04	7.89%

2) Analytical Sensitivity

The sensitivity is defined as the concentration of FK506 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 2ng/ml.

3) Specificity

The specificity of the FK506 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
FK520	500ng/ml	0.3%

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
15.332ng/ml	14.429ng/ml	94%

5) Linearity

Use FK506 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	2	r=0.9834
C	4	
D	10	
E	25	
F	50	

6) Method comparison

A comparison of MAGLUMI FK506 (y) with a commercially available FK506 test (x) using clinical samples gave the following correlations (ng/ml):

Linear regression
y = 0.97x+50.0
r = 0.982
Sy.x = 22

Number of samples measured: 150
The sample concentrations were between 8 and 45 ng/ml.

REFERENCES

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