Snibe Diagnostic

CE REF 130257003M:100 tests/kit
130657003M: 50 tests/kit
130757003M: 30 tests/kit

MAGLUMI® Tacrolimus (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Tacrolimus in human whole blood using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the management of transplant patients receiving tacrolimus therapy.

SUMMARY

Tacrolimus is a 23-membered macrolide lactone. It is a neutral and hydrophobic compound that crystallizes as colorless prisms. Its molecular formula is $C_{44}H_{69}NO_{12}$, and its relative molecular mass is 803[1]. Tacrolimus (FK506) is a potent immunosuppressant widely used for organ transplantation patients[2] . Tacrolimus was associated with a significant reduction in acute, refractory acute, and chronic rejection episodes[3]. In bone marrow transplant (BMT) recipients, the incidence of tacrolimus grade II–IV graft-versus-host disease was significantly lower with tacrolimus than cyclosporin treatment[4].

The mechanism of action of tacrolimus is similar to that of cyclosporine, even though their chemical structures differ greatly. Both tacrolimus and cyclosporine can be considered prodrugs, because they exert immunosuppressive properties only when bound to their respective immunophilin target molecules. The immunophilin-drug complex binds competitively to and inhibits calcineurin, a phosphatase whose activity is dependent on its being bound to calcium and calmodulin. Inhibition of calcineurin is believed to mediate the immunosuppressive activity of both tacrolimus and cyclosporine. Inhibition of calcineurin activity by tacrolimus inhibits transcription. Tacrolimus also inhibits the transcription of genes that encode interleukin-3, interleukin-4, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-α, and γ-interferon—cytokines involved in the early phase of T-cell activation. The inhibition of gene expression for interleukin-2 by tacrolimus is most notable, since the activation of this gene is crucial for growth and proliferation of cytotoxic T cells. Tacrolimus can also enhance in T cells the degradation of messenger RNA for interleukin-2 and granulocyte-macrophage colony-stimulating factor[5].

One of the main limitations for the use of the immunosuppressive drugs in clinical practice is the association of major and unpredictable interindividual variations in their pharmacokinetics, which leads to variations in drug exposure and a number of dose-related side effects[6]. Principle adverse effects associated with tacrolimus include nephrotoxicity, neurotoxicity, diabetogenesis, gastrointestinal disturbances, hypertension, infections, malignant complications and can cause insomnia, alopecia, nausea and pruritus in some patients. Adverse events tend to occur the most frequently in the first few months after transplantation and decline thereafter, possibly in line with reductions in tacrolimus concentrations. Nephrotoxicity, neurotoxicity, diabetogenesis, gastrointestinal disturbances and infections occur more frequently or are more severe at higher concentrations[7–9].

While tacrolimus is a potent immunosuppressive drug, it has a narrow therapeutic index. Monitoring tacrolimus blood concentrations provides the clinician with information of predictive value for managing the risk of nephrotoxicity and acute rejection in liver transplant patients. Routine monitoring of tacrolimus blood concentrations must be used in conjunction with appropriate clinical evaluation of the patient to optimize immunosuppressive therapy[10].

TEST PRINCIPLE

Competitive chemiluminescence immunoassay.

The pretreated sample, Displacing Reagent, ABEI labeled with Tacrolimus monoclonal antibody are mixed thoroughly and incubated, buffer and magnetic microbeads coated with Tacrolimus antigen conjugate are then added and incubated. Tacrolimus present in the sample compete with Tacrolimus antigen immobilized on the magnetic microbeads for binding Tacrolimus monoclonal antibody labeled with ABEI, forming immuno-complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is inversely proportional to the concentration of Tacrolimus present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with Tacrolimus antigen conjugate (~8.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of Tacrolimus antigen in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of Tacrolimus antigen in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Displacing Reagent	Sodium deoxycholate.	3.5 mL	2.0 mL	1.4 mL
Buffer	PBS buffer, NaN_3 (<0.1%).	6.5 mL	4.0 mL	3.0 mL
ABEI Label	ABEI labeled with Tacrolimus monoclonal antibody (~125 ng/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	6.5 mL	4.0 mL	3.0 mL
Control 1	A low concentration of Tacrolimus antigen (5.00 ng/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of Tacrolimus antigen (20.0 ng/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Whole Blood Pretreatment Reagent	NH_4Cl (~8.30 mg/mL).	4.0 mL	2.0 mL	1.5 mL
All reagents are provided ready-to-use.				

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 10-30°C	6 hours
Opened at 2-8°C	6 weeks
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Type	Collection Tubes
Whole blood	K2-EDTA, K3-EDTA

- 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. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Sample material: whole blood. Collect blood aseptically following the universal precautions for venipuncture.
- Do not use heat-inactivated samples and specimens with obvious microbial contamination.
- To prevent cross contamination, use of disposable pipettes or pipette tips are recommended.

Preparation for Analysis

- Mix each sample thoroughly by slow inversion of the container 5-10 times before use. Older whole blood specimens may take a longer mixing time. Visual inspection is recommended to assure the specimen is adequately mixed. Do not vortex as this may cause foaming.
- Follow the steps listed below to pretreat specimens:
  - Precision pipette 1 mL of each whole blood sample from EDTA tube into a centrifuge tube immediately after mixing.
- Add 20 μL of Whole Blood Pretreatment Reagent to the centrifuge tube.
  - Cap the centrifuge tube and vortex immediately 2 min (2000 rpm).
  - It is recommended that the pretreated sample should be tested immediately. If not, it may be stored for up to 3 days at 2-8 °C.
- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- The sample volume required for a single determination of this assay is 40 μL.

Specimen Storage

Specimens before pretreating may be stored up to 8 hours at 10-30°C or 7 days at 2-8°C, or 6 months frozen at -20°C. Frozen specimens subjected to up to one freeze/thaw cycle have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, with Tacrolimus concentrations above the analytical measuring interval, can be diluted with manual dilution procedure. Samples must be diluted before pretreatment. The recommended dilution ratio is 1:2. The concentration of the diluted sample must be >25.0 ng/mL.
- For manual dilution, multiply the result by the dilution factor.
- Please choose applicable diluents or ask Snibe for advice before manual dilution.

PROCEDURE

Materials Provided

Tacrolimus (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6 or Integrated System Biolumi 8000, Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the

quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

Traceability: This method has been standardized against the USP reference standard (Catalog number: 1642802).

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 28 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[11].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the Tacrolimus assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi systems and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order Tacrolimus (CLIA) Controls (REF: 160201485MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the Tacrolimus 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 Analyzer Operating Instructions.

Interpretation of Results

No firm therapeutic range exists for tacrolimus in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of tacrolimus, co-administration of other immunosuppressants, type of transplant, time post-transplant and a number of other factors contribute to different requirements for optimal blood levels of tacrolimus. Therefore, individual tacrolimus values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Each user must establish his or her own ranges based on clinical experience.

Therapeutic ranges vary according to the commercial test used, and therefore should be established for each commercial test. Values obtained with different assay methods cannot be used interchangeably due to differences in assay methods and cross-reactivity with metabolites, nor should correction factors be applied. Therefore, consistent use of one assay for individual patients is recommended.

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the Tacrolimus results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[12].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n = 180). The following results were obtained:

Sample	Mean (ng/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (ng/mL)	%CV	SD (ng/mL)	%CV	SD (ng/mL)	%CV
Whole Blood Pool 1	5.118	0.154	3.01	0.089	1.74	0.249	4.87
Whole Blood Pool 2	10.110	0.225	2.23	0.162	1.60	0.388	3.84
Whole Blood Pool 3	20.320	0.319	1.57	0.109	0.54	0.545	2.68
Control 1	5.009	0.161	3.21	0.041	0.82	0.202	4.03
Control 2	20.047	0.309	1.54	0.146	0.73	0.633	3.16

Linear Range

0.500-50.0 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.300-100 ng/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.100 ng/mL.

Limit of Detection (LoD) =0.300 ng/mL.

Limit of Quantitation (LoQ) =0.500 ng/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Hemoglobin	1000 mg/dL	Erythromycin	20 mg/dL
Intralipid	1500 mg/dL	Fluconazole	30 μg/mL
Bilirubin	60 mg/dL	Flucytosine	40 μg/mL
HAMA	40 ng/mL	Ganciclovir	1000 μg/mL
ANA	398 AU/mL	Itraconazole	50 μg/mL

Rheumatoid factor	500 IU/mL	Kanamycin	100 μg/mL
Biotin	0.5 mg/dL	Ketoconazole	50 μg/mL
Cholesterol	500 mg/dL	Lidocaine	6 mg/dL
Human albumin	12 g/dL	Mycophenolic Acid Glucuronide	1800 μg/mL
Uric acid	20 mg/dL	Mycophenolic Acid	500 μg/mL
IgG	12 g/dL	Phenobarbital	15 mg/dL
K2-EDTA	22.75 μmol/mL	Sirolimus	60 ng/mL
K3-EDTA	22.75 μmol/mL	Tobramycin	2 mg/dL
Amphotericin B	5.8 μg/mL	Trimethoprim	40 μg/mL
Human γ-Globulin	12 g/dL	Vancomycin	6 mg/dL
Cyclosporine	5000 ng/mL		

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactant in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
M I	50 ng/mL	M VI	50 ng/mL
M III	50 ng/mL	M VII	50 ng/mL
M IV	50 ng/mL	M VIII	50 ng/mL

Method Comparison

A comparison of the Tacrolimus assay with a commercially available immunoassay, gave the following correlations (ng/mL):

Number of samples measured: 118

Passing-Bablok: $\hat{y}$=0.9972*x* - 0.0052, τ=0.958.

The clinical specimen concentrations were between 0.513 and 49.91 ng/mL.

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
	Temperature limit (Store at 2-8°C)		Use-by date
	Contains sufficient for<n> tests		Keep away from sunlight
	This way up		Authorized representative in the European Community
	*In vitro* diagnostic medical device		Kit component
	Catalogue number		Batch code
	CE marking		

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