



130257001M: 100 tests/kit
 130657001M: 50 tests/kit
 130757001M: 30 tests/kit

MAGLUMI® Cyclosporine (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Cyclosporine (CSA) 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 cyclosporine therapy.

SUMMARY

Cyclosporine (CSA) is a cyclic polypeptide immunosuppressant agent consisting of 11 amino acids. It is produced as a metabolite by the fungus species *Beauveria nivea*¹. CSA becomes active after complexing with cyclophilin A and B. These complexes would exert inhibitory effects in T cell receptor-stimulated transcription genes and cell-activated pathways^{2,3}. The inhibition of calcineurin restricts the dephosphorylation and nuclear translocation of nuclear factor of activated T cells (NFAT), which regulates transcription of several cytokines, including IL-2, IL-4, TNF- α , and interferon- γ , and therefore limits lymphocyte activation and proliferation⁴.

CSA is a potent immunosuppressant drug that is used in the prevention of allograft rejection^{5,6}. With the introduction of cyclosporine in kidney, heart, liver, pancreas and bone marrow transplantation, the survival rate of transplant patients has been significantly increased^{7,8}. CSA half-life in serum ranges between 6 and 24 hours, and measurements at 6 and 14 hours, as well as average concentrations, were all highly predictive; its oral bioavailability and systemic clearance are controlled by the cytochrome P450 and the efflux p-glycoprotein pump, a trans-membrane transporter expressed in the gastrointestinal tract and in the liver^{2,3}. The dosage of CSA is complicated by the large intra- and interindividual variability in its pharmacokinetics, as well as by the narrow concentration range between insufficient immunosuppression and toxicity⁹. Inadequate cyclosporine concentrations might result in rejection of the transplanted organ^{9,10}. High levels may lead to severe adverse effects. The main adverse reaction is nephrotoxicity and hepatotoxicity. Other side effects include gum hyperplasia, tremor, high blood pressure and hirsutism^{11,12}. Therapeutic drug monitoring (TDM) plays a key role in helping clinicians maintain blood and plasma levels of immunosuppressive drugs within their respective therapeutic ranges¹⁰. TDM of cyclosporine has been accepted as an essential tool in the management of transplant recipients. The availability of simple, sensitive assays that measure amounts of parent drug in blood samples permits the use of whole-blood cyclosporine concentrations to individualize dosing regimens according to pharmacokinetic principles. The most widely used index is the predose, or trough, concentration (Co)¹³.

TEST PRINCIPLE

Competitive chemiluminescence immunoassay.

The pretreated sample, ABEI labeled with CSA monoclonal antibody, buffer and magnetic microbeads coated with CSA antigen conjugate are mixed thoroughly and incubated. CSA present in the sample compete with CSA antigen immobilized on the magnetic microbeads for binding CSA 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 CSA present in the sample.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with CSA antigen conjugate (~8.00 μ g/mL) in PBS buffer, NaNa ₃ (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of CSA antigen in PBS buffer, NaNa ₃ (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Calibrator High	A high concentration of CSA antigen in PBS buffer, NaNa ₃ (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Buffer	Tris-HCl buffer, NaNa ₃ (<0.1%).	5.5 mL	3.5 mL	2.7 mL
ABEI Label	ABEI labeled with CSA monoclonal antibody (~0.250 μ g/mL) in Tris-HCl buffer, NaNa ₃ (<0.1%).	10.5 mL	6.0 mL	4.2 mL
Control 1	A low concentration of CSA antigen (100 ng/mL) in PBS buffer, NaNa ₃ (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Control 2	A high concentration of CSA antigen (400 ng/mL) in PBS buffer, NaNa ₃ (<0.1%).	1.5 mL	1.5 mL	1.5 mL
Whole Blood Pretreatment Reagent	NH ₄ Cl (~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 handling 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 μ L 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 80 μ L.

Specimen Storage

Specimens before pretreating may be stored up to 24 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 CSA 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 >1000 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

Cyclosporine (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 1158504.

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 guidelines¹⁴.

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 Cyclosporine 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 Cyclosporine (CLIA) Controls (REF: 160201486MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

The analyzer automatically calculates the CSA 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.

Conversion factors: $\text{ng/mL} \times 1 = \mu\text{g/L}$, $\text{ng/mL} \times 0.8315 = \text{nmol/L}$

Interpretation of Results

No firm therapeutic range exists for cyclosporine in whole blood. The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic effects of cyclosporine, coadministration of other immunosuppressants, type of transplant, time post-transplant, and a number of other factors contribute to different requirements for optimal blood levels of cyclosporine. Therefore, individual cyclosporine values cannot be used as the sole indicator for making changes in the treatment regimen and each patient should be thoroughly evaluated clinically before treatment adjustments 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 CSA results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies^{15,16}. Additional information may be required for diagnosis.
- 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 observed¹⁷.
- 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	98.670	3.116	3.16	0.990	1.00	4.549	4.61
Whole Blood Pool 2	399.291	9.589	2.40	1.609	0.40	12.236	3.06
Whole Blood Pool 3	997.133	14.201	1.42	9.270	0.93	27.538	2.76
Control 1	97.433	3.182	3.27	1.528	1.57	4.181	4.29
Control 2	397.890	7.515	1.89	6.213	1.56	11.018	2.77

Linear Range

30.0-2000 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

20.0-4000 ng/mL (defined by the Limit of Detection and the maximum of the master curve*Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) = 10.0 ng/mL.

Limit of Detection (LoD) = 20.00 ng/mL.

Limit of Quantitation (LoQ) = 30.0 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 $\pm 10\%$. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Hemoglobin	1000 mg/dL	Human γ -Globulin	12 g/dL
Intralipid	1500 mg/dL	Kanamycin	100 $\mu\text{g/mL}$
Bilirubin	60 mg/dL	Ketoconazole	50 $\mu\text{g/mL}$
HAMA	40 ng/mL	Lidocaine	6 mg/dL
ANA	398 AU/mL	Mycophenolic Acid Glucuronide	1800 $\mu\text{g/mL}$
Rheumatoid factor	2000 IU/mL	Mycophenolic acid	500 $\mu\text{g/mL}$
Cholesterol	500 mg/dL	Phenobarbital	15 mg/dL
Human albumin	12 g/dL	Sirolimus	60 ng/mL
Uric acid	20 mg/dL	Spectinomycin	100 $\mu\text{g/mL}$
IgG	12 g/dL	Tacrolimus	60 ng/mL

K2-EDTA	22.75 $\mu\text{mol/mL}$	Tobramycin	2 mg/dL
K3-EDTA	22.75 $\mu\text{mol/mL}$	Vancomycin	6 mg/dL
Biotin	0.5 mg/dL		

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 $\pm 10\%$. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
AM1	2000 ng/mL	AM19	2000 ng/mL
AM1c	2000 ng/mL	AM4n	2000 ng/mL
AM9	2000 ng/mL	AM1c9	2000 ng/mL

Method Comparison

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

Number of samples measured: 109

Passing-Bablok: $\hat{y} = 1.0035x - 2.3589$, $r = 0.982$.

The clinical specimen concentrations were between 31.43 and 1995 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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