1. INTENDED USE
The kit has been designed for the quantitative determination of FK 506 in whole blood. The method can be used for samples over the range of 0.50 ng/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST
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. 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 tacrolimus is under active investigation. Tacrolimus binds to a family of proteins termed FK506 (tacrolimus) binding proteins (FKBPs). The formation of a larger pentameric complex comprised of FKBP, tacrolimus, 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.

Tacrolimus may be administered IV or orally. Absorption from gastrointestinal tract is variable and irregular. Pharmacokinetic studies with tacrolimus 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 tacrolimus. Tacrolimus is bound to proteins, mainly albumin, and alpha-1-acid glycoprotein, and is highly bound to erythrocytes. The distribution of tacrolimus 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).

Tacrolimus is extensively metabolized in the liver and small intestine microsomes utilizing the cytochrome P-450 enzymes. Nine different metabolites of tacrolimus have been identified; several of the metabolites have been found and tested in whole blood.

The use of tacrolimus is associated with serious toxic side effects, primarily nephrotoxicity. At the present time it is not clear whether the nephrotoxicity of tacrolimus is the result of parent drug, metabolites, or a combination of both. Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea.

3. PRINCIPLE OF THE TEST
Competitive immunoluminometric assay;
Use anti-FK 506 monoclonal antibody to label FITC, and purified FK506 antigen to label ABEI. Sample, Calibrator or Control, FITC label, and magnetic microbeads are mixed thoroughly and incubated at 37°C, then add the ABEI label, forming 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.

4. KIT COMPONENTS
4.1 Material supplies

<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads : TRIS buffer, 1.2%(W/V), 0.2%NaNO₃, coated with anti-FK 506 monoclonal antibody</td>
</tr>
<tr>
<td>Calibrator Low (Lyophilize)</td>
</tr>
<tr>
<td>Calibrator High (Lyophilize)</td>
</tr>
<tr>
<td>ABEI Label: purified FK506 antigen labeled ABEI, containing BSA, 0.2%NaNO₃</td>
</tr>
<tr>
<td>FITC Label: anti-FK506 monoclonal antibody labeled FITC, containing BSA, 0.2%NaNO₃</td>
</tr>
<tr>
<td>Displacing reagent</td>
</tr>
<tr>
<td>Treat Buffer</td>
</tr>
<tr>
<td>Red Cell Lysate</td>
</tr>
</tbody>
</table>

Except Calibrators, other reagents are provided ready-to-use.

- The calibrators need to be dissolved with the distilled water manually; the volume of the distilled water can refer to the reagent label.
- Place the Calibrator glass vials into the integral to their position. Calibrator Low at the Second hole and Calibrator High at the Third hole. Make sure they are fixed with the integral. If they are loosen for the hole, use some soft material to fill the gap between the glass and
the integral hole.

- If the kit will be used for a long time, store the calibrator Low and High at -20°C; they can be frozen repeatedly.

The steps of preparing the whole blood, please refer to Chapter 7

**Accessories required but not provided**

- Maglumi Reaction module
- Maglumi Starter kit 1+2
- Maglumi Light check
- Maglumi Wash / System Liquid

4.2 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 mins. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

**Do not interchange magnetic microbeads from different lots.**

4.3 Storage of the Reagents Integral

- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. After this period, it is still possible to keep on using the Reagent Integral provided that the controls are found within the expected ranges.
- Keep upright for storage.
- Keep away from direct sunlight.

### 5. ORIGIN OF CALIBRATORS

Calibrators in the reagent kit are from Sigma.

Biological root: synthetic materials, processed by HPLC purification, with a purity ≥97%. No HbsAg, anti-HCV, and anti-HIV is found.

### 6. CALIBRATION

#### 6.1 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.

#### 6.2 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.
- Room temperature changes exceed 5°C (recommendation).

7. SAMPLE COLLECTION, MATERIAL AND STORAGE

**Whole blood**

Collect 5ml of venous blood in a glass tube without additives. Add 50μl Red Cell Lysate to each tube. If you collect 2ml of venous blood in a tube, then add 20μl Red Cell Lysate to the tube, following the ratio.

The whole blood can be stable for 12 hours at 2-8°C

### 8. WARNING AND PRECAUTIONS FOR USERS

- For use in IN-VITRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labelled expiry date.
- 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 24,1990,p.2143). A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- 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

### 9. TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto Analyzer. Each test parameter is indentified via a RFID tag on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

<table>
<thead>
<tr>
<th>Auto dilution</th>
<th>Whole blood</th>
<th>Treat buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>+100ul</td>
<td>+100ul</td>
<td>+40ul</td>
</tr>
<tr>
<td>+20ul</td>
<td>+40ul</td>
<td>FITC label</td>
</tr>
<tr>
<td>+50ul</td>
<td>+20ul</td>
<td>Displacing reagent</td>
</tr>
<tr>
<td>+50ul</td>
<td>+50ul</td>
<td>Microbeads</td>
</tr>
<tr>
<td>15min incubation</td>
<td>+40ul</td>
<td>ABEI label</td>
</tr>
<tr>
<td>15 min</td>
<td>Incubation</td>
<td>400μl each time Cycle washing</td>
</tr>
<tr>
<td>3 s</td>
<td>Measurement</td>
<td></td>
</tr>
</tbody>
</table>

### 10. QUALITY CONTROL

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

### 11 RESULTS

#### 11.1 Calculation of Results

The analyzer automatically calculates the FK 506 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 Fully Auto Operator’s Manual.

#### 11.2 Interpretation of Results

Reference values:

- The complexity of the clinical state and individual differences in sensitivity to the immunosuppressive effects of FK 506 will cause different requirements for optimal whole blood levels of FK 506. Each patient should be thoroughly evaluated clinically before treatment adjustments are made. The physician should establish individual patient ranges based on these clinical evaluations. Individual whole blood FK 506 values cannot be used as the sole indicator for making changes in the treatment regime.
- We suggest that the effective concentration of the FK506 in the whole blood is 3.0 ng/ml-39.4 ng/ml
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.
- Test results need NOT to multiply dilution rate!

#### 12. LIMITATIONS OF THE PROCEDURE

- Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
- A skilful 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.

### 13. PERFORMANCE CHARACTERISTICS

#### 13.1 Accuracy

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluent, 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%.

#### 13.2 Sensitivity

The sensitivity is defined as the concentration of FK 506 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 2 ng/ml.

#### 13.3 Specificity

The specificity of the FK 506 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When FK520=500.0ng/ml, the detection result of FK 506 is less than 10 ng/ml.

#### 13.4 Linearity

Conduct a logarithmic transform to the RLU value and concentration value of 8 standards. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

### 14 References

2. Porayko MK, Gonwa TA, Klintmalm GB, et al. Comparing...


