1. INTENDED USE
The kit has been designed for the quantitative determination of Cortisol in human serum.
The method can be used for samples over the range of 0-600ng/ml.
The test has to be performed on the Maglumi fully auto analyzer (including

2. SUMMARY AND EXPLANATION OF THE TEST
Cortisol is the most potent glucocorticoid produced by the human adrenal.
It is synthesized from cholesterol and its production is stimulated by pituitary
adrenocorticotropic hormone (ACTH) which is regulated by corticotropin
releasing factor (CRF). ACTH and CRF secretions are inhibited by high
cortisol levels in a negative feedback loop. In blood a majority of cortisol is
bound with high affinity to corticosteroid binding globulin (CBG). Cortisol acts
through specific intracellular receptors and affects numerous physiologic
systems including immune function, glucose counter regulation, vascular tone,
and bone metabolism.
The body level of cortisol in the bloodstream displays what is known as a
diurnal variation therefore, normal concentrations of cortisol vary throughout a
24-hour period. Cortisol levels in normal individuals are highest in the early
morning at around 6-8 am and are lowest around midnight.
The factor controlling this rhythm is not completely defined and can be
interrupted by a number of physical and psychological conditions. ACTH and
cortisol are secreted independent of circadian rhythm in response to physical
and psychological stress. Elevated cortisol levels and lack of diurnal variation
have been identified with Cushing's disease (ACTH hypersecretion).
Elevated circulating cortisol levels have also been identified in patients with adrenal
tumors. Low cortisol levels are found in primary adrenal insufficiency (e.g.
adrenal hypoplasia, Addison’s disease) and in ACTH deficiency. Due to the
normal circadian variation in cortisol levels, distinguishing normal from
abnormally low cortisol levels can be difficult, therefore several daily
collections are recommended. Additional stress tests such as ACTH
stimulation or dexamethasone suppression aids in the diagnosis of adrenal
related disease. The measurement of urinary cortisol levels is of value in the
diagnosis of Cushing's Syndrome. For this test, urine is collected over a
24-hour period and analyzed.

3. PRINCIPLE OF THE TEST
Competitive immunoluminometric assay;
Use a purified Cortisol antigen to label ABEI, and use an anti-Cortisol
monoclonal antibody labeled FITC. Sample, Calibrator, or Control, FITC
Label, ABEI Label, and magnetic microbeads coated with anti-FITC
complexes; after sediment in a magnetic field, decant the supernatant, then
mix the antibody-antigen complexes; after sediment in a magnetic field, decant the supernatant, then
place the Integral turn the small wheel of the magnetic microbeads compartment to and fro,
before the sealing is removed, gentle and careful horizontal shaking of the

4. KIT COMPONENTS
4.1 Material supplies

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Integral for 100 determinations</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Lot number</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Catalogue Code</td>
<td>10.5ml</td>
</tr>
<tr>
<td>Expiry date (Use by…</td>
<td>10.5ml</td>
</tr>
<tr>
<td>Temperature limitation ( store at 2…8 °C)</td>
<td>All reagents are provided ready-to-use.</td>
</tr>
<tr>
<td>Number of tests</td>
<td>Accessories required but not provided</td>
</tr>
<tr>
<td>Keep away from sunlight</td>
<td>Maglumi Reaction module</td>
</tr>
<tr>
<td>Biological risks</td>
<td>Maglumi Starter kit 1+2</td>
</tr>
</tbody>
</table>

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 re-suspended.

**Do not interchange Nano Magnetic Microbeads from different reagents!**

### 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 identified within the expected ranges.
- Keep up 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 ≥ 98%. 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 2 weeks 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.

### 7. Sample Collection, Material and Storage
- Collect samples using standard procedures.
- Sample material: serum.
- Store at 2-8°C 24 hours.
- For longer storage periods: freeze to below - 20°C.
- 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) If plasma sample is needed, EDTA tube is confirmed has no interference.
- (c) Liquaemin Sodium tube is found to increase the sample RLU and cause test results deviation.
- (d) Please ask SNIBE for advice if special additive must be used in the sample blood.

## 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 labeled 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 24,2000,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 identified via a RFID tag on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

| 40μl | Sample, calibrator or controls |
| 80μl | ABEI Label |
| 80μl | FITC Label |
| 20μl | Nano magnetic microbeads |

**15 min** Incubation

**400μl each time** Cycle washing

**3 s** Measurement

### 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 Cortisol 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:** Normal adult: 52-360 ng/ml
- 08:30am-09:30am 72.6-322.8 ng/ml
- 16:30pm-17:30pm 32.4-150.0 ng/ml
- **Results may differ between laboratories due to variations in population and test method.** Each laboratory should establish its own reference range.

## 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 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.

## 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 Precision
Intra-assay coefficient of variation was evaluated on Calibrator High repeatedly measured 10 times in the same assay, calculating their coefficient of variation, the results should be ≤10% Inter-assay coefficient of variation was evaluated on three batches of kit, repeatedly measured 10 times of Calibrator High, calculating three batches of kit for Calibrator High between the measured values of the coefficients of variation, the results should ≤15%

### 13.3 Sensitivity
The sensitivity is defined as the concentration of Cortisol 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 2.5ng/ml.

### 13.4 Specificity
The specificity of the Cortisol assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When testosterone=100 ng/ml, the detection result of Cortisol is less than 0.03 ng/ml; When progesterone=100ng/ml, the detection result of Cortisol is less than 0.03 ng/ml; When androstenedione=100ng/ml, the detection of Cortisol is less than 0.03ng/ml

### 13.5 Linearity
Conduct a logarithmic transform to the RLU value and concentration value of 6 standards. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

### 14. References