1. INTENDED USE
The kit has been designed for the quantitative determination of Adrenocorticotropic Hormone (ACTH) in human serum or plasma. The method can be used for samples over the range of 0-2000 pg/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
Adrenocorticotropic hormone (ACTH) is a polypeptide hormone which exists principally as a chain, 39 amino acids long, with a molecular mass of approx. 4500 daltons. It is produced in the pituitary and serves to stimulate steroid production by the adrenal cortex. ACTH secretion is in turn controlled by the hypothalamic hormone corticotrophin releasing factor (CRF) and by negative feedback from cortisol.

ACTH determinations are valuable in the differential diagnosis of adrenal insufficiency and hypersecretion. In Addison’s disease (primary adrenal insufficiency), elevated levels are typical, whereas low levels are the rule when adrenal insufficiency is secondary to pituitary dysfunction. ACTH determinations can also help to identify the cause of cortisol hypersecretion in Cushing’s syndrome. ACTH levels are typically low when this is due to lesions or hyperplasia of the adrenal cortex, and high when it is due to ectopic ACTH production or hypersecretion of ACTH by the pituitary.

Plasma levels of ACTH exhibit a significant diurnal variation. It is important, therefore, to standardize the time of collection: reference ranges have typically been established for approximately 9 in the morning.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay;
Use an anti-ACTH monoclonal antibody to label ABEI, and use another monoclonal antibody to label microbeads. Sample, Calibrator, or Control, ABEI Label and magnetic microbeads coated with monoclonal antibody are mixed thoroughly and incubated at 37 °C, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of ACTH present in controls or samples.

4. KIT COMPONENTS
4.1 Material supplies
Reagent Integral for 100 determinations
Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN₃, coated with sheep anti- ACTH monoclonal antibody 2.5ml
Calibrator low 3.0ml
Calibrator high 3.0ml
ABEI Label: anti-ACTH monoclonal antibody labeled ABEI contains BSA, 0.2%NaN₃. 12.5ml

All reagents are provided ready-to-use.

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.

4.3 Storage of the Reagents Integral
Do not interchange nano magnetic microbeads from different reagents.

4.4 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 lot: synthetic materials, processed by HPLC purification, with a purity ≥95%. 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 1 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 or plasma.
- 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).

Serum:
- elbow vein blood 5ml in the tube, centrifugation at room temperature, serum was separated and stored at 2°C -8°C.
- Serum samples were stable for 12 hours at 2-8°C. If preserved more than 12 hours, please pack, -20°C can be stored for 30 days.
- to avoid repeated freezing and thawing

Plasma:
- elbow vein blood 5ml in the tube, then add EDTA anticoagulant, centrifuged and separated plasma stored at2-8°C.
- Plasma was stable at 2-8°C for 12 hours. If preserved more than 12 hours, please packed, -20°C can be stored for 30 days.
- to avoid repeated freezing and thawing

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 indentified via a barcode on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

| 200μl | Sample, calibrator or controls |
| +100μl | ABEI Label |
| +50μl | Nano magnetic microbeads |
| 30 min | Incubation |
| 40μl 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 ACTH 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 pg/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11.2 Interpretation of Results

- Reference values:
  - Serum am 8:00-10:00, 6-40 pg/ml
  - pm 4:00, 3-30 pg/ml
  - pm 12:00, <20 pg/ml
- Plasma (We don’t suggest the customer to test with the plasma, the values below are only for reference)
  - am 8:00-10:00, 213-639 pg/ml
  - pm 4:00, 124-642 pg/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

12.1 Patients with malignancies may exhibit ACTH values within the normal range. ACTH concentrations may be elevated in case of liver cirrhosis, hepatitis or tyrosinaemia. Thus, ACTH determination is more suitable for therapeutic monitoring and follow-up as well as for a comparison with histological results. ACTH serum levels may only be interpreted in context with the clinical picture and other diagnostic procedures. The ACTH assay should not be used as the only criterion for cancer screening.

12.2 HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

12.3 High-Dose Hook

No high-dose hook effect was seen for ACTH concentrations up to 50,000 pg/ml.

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 at 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 of the assay defined as the concentration of H-ALB 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 3 pg/ml.

13.4 Specificity

The specificity of the ACTH assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When BSA=50 μg/ml, the detection result of ACTH< 2 pg/ml.

13.5 Linearity

Conduct a log-linear transformation 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