1. INTENDED USE
The kit has been designed for the quantitative determination of Alpha-Fetoprotein (AFP) in human serum. The method can be used for samples over the range of 0-1000 IU/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
Alpha-fetoprotein (AFP) is a glycoprotein with a high molecular weight (approx. 68,000 D) consisting of a single polypeptide chain. AFP, which belongs to the group of oncofetal proteins, is produced by the yolk sac and in the fetal liver.

In oncology, AFP is determined in patients with liver-cell carcinoma or germ-cell tumors (non-seminomatous tumors of the testes; endodermal sinus tumor of the ovaries). AFP plays an important role for pregnancy monitoring, too. During pregnancy, AFP levels in maternal blood continuously increase. Between weeks 28 to 32, a maximum is reached: after this period, a decrease can be observed until delivery. In the amniotic fluid, the maximum is already achieved between the 13th and 15th week of gestation. Elevated AFP levels in early pregnancy indicate neural tube defects (spina bifida, anencephaly). Lower AFP concentrations in maternal serum are indicative of Down’s syndrome.

The determination of serum AFP during therapeutic monitoring provides valuable information about the success or failure of treatment as well as about the occurrence of recidivation.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay:
Use an anti-AFP monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator or Control, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37 °C and cycle washing for 1 time. Then add ABEI Label, incubation and form a sandwich, then washing for the 2nd 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 AFP 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-FITC polyclonal antibody. 2.5ml
- Calibrator low 2.5ml
- Calibrator high 2.5ml
- ABEI Label: anti-AFP monoclonal antibody labeled ABEI, contains BSA, 0.2%NaN₃. 22.5ml
- FITC Label: anti-AFP monoclonal antibody labeled FITC, contains BSA, 0.2%NaN₃. 12.5ml
- Diluent: 25ml
All reagents are provided ready-to-use.

*Please prepare 0.9% sodium chloride solution in case of insufficient diluents.

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 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 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 Fitzgerald.

Biological root: extracted from human serum, handled by means of SDS PAGE 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 recalibration.

6.2 Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- After 4 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 conformed to have no effect on the results RLU.
  - (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. Interfering Substances

No interference with test results is seen by concentrations of bilirubin < 0.125mg/ml, haemoglobin < 16mg/dl or triglycerides < 12.5mg/ml.

9. 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 prior to disposal. The disposal of biohazardous waste should therefore be conducted in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory.
- Keep away from direct sunlight.

10. 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 |
| +100μl | FITC label |
| +20μl | Nano magnetic microbeads |
| 10 min | Incubation |
| 400μl each time | Cycle washing |
| ≤200μl | ABEI label |
| 10 min | Incubation |
| 400μl each time | Cycle washing |

11. Quality Control

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

12. Results

12.1 Calculation of Results

The analyzer automatically calculates the AFP 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 IU/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

- Conversion factor: 1 ng/ml = 0.83 IU/ml

12.2 Interpretation of Results

- Reference values: < 0.5 IU/ml
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

13. Limitations of the procedure

13.1 Patients with malignancies may exhibit AFP values within the normal range. AFP concentrations may be elevated in case of liver cirrhosis, hepatitis or tyro sineaemia. Thus, AFP determination is more suitable for therapeutic monitoring and follow-up as well as for a comparison with histological results.

- AFP serum levels may only be interpreted in context with the clinical picture and other diagnostic procedures. The AFP assay should not be used as the only criterion for cancer screening.

- HAMA Patients sample 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.

14. Performance Characteristics

14.1 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%.

14.2 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 < 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%.

14.3 Sensitivity

The sensitivity is defined as the concentration of AFP 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 1.25 IU/ml.

14.4 Specificity

The specificity of the AFP assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When CEA=200 IU/ml, the detection results of AFP <0.5 IU/ml; When CA125=200 IU/ml, the detection results of AFP <0.5 IU/ml; When CA153=200 IU/ml, the detection results of AFP <0.5 IU/ml.

14.5 Linearity

Conduct a logarithmic transform to the RLU value and concentration value of 5 standards. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

15. References