1. INTENDED USE
The kit has been designed for the quantitative determination of Cancer Antigen 15-3 (CA15-3) in human serum. The method can be used for samples over the range of 0-1000IU/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
The tumour associated antigen CA 15-3 bears the epitopes of two different monoclonal antibodies Mab 115D8 and Mab DF3. Mab 115D8 was raised against the surface antigen MAM-6 (MW 400,000) which is found on mammary epithelial cell and most breast carcinoma cells. Mab DF3 was raised against membrane antigens of mammary carcinoma cells. The monoclonal antibody reacts with an epitope termed DF3 on a glycoprotein with the molecular weight 290,000. Elevated CA 15-3 assay values are found in patients with breast carcinoma. Due to its high sensitivity for metastatic breast cancer, determination of CA 15-3 assay values is particularly important in the diagnosis of relapses and in monitoring relapse therapy.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay; Use an anti-CA153 monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator, Control, ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC 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 CA153 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-CA153 monoclonal antibody labeled ABEI, contains BSA, 0.2%NaN3. 10.5ml
  - FITC Label: anti-CA153 monoclonal antibody labeled FITC, contains BSA, 0.2%NaN3. 10.5ml
- 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. 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 Biodesign. Biological root: human tissue fluid. 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 conformed has no effect on the results RLUs.
  - (c) Li-Deaemmin 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 <0.125 mg/ml, haemoglobin <16 mg/dl or triglycerides <12.5 mg/ml.

9. WARNING AND PRECAUTIONS FOR USERS
- For use 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. Disposal 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.

10. Test Procedure
To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto analyzer. Each test parameter is 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 | ABCI Label |
| +80μl | FITC Label |
| +20μl | Nano magnetic microbeads |
| 15 min | Incubation |
| 400μl each time | Cycle washing |
| 3 sec | Measurement |

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 CA153 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.

12.2 Interpretation of Results
- Reference values: < 30 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 CA153 values within the normal range. CA153 concentrations may be elevated in case of liver cirrhosis, hepatitis or tyrosinaemia. Thus, CA153 determination is more suitable for therapeutic monitoring and follow-up as well as for a comparison with histological results. CA153 serum levels may only be interpreted in context with the clinical picture and other diagnostic procedures. The CA153 assay should not be used as the only criterion for cancer screening.

13.2 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.

13.3 High-Dose Hook
No high-dose hook effect was seen for CA153 concentrations up to 10,000 IU/ml.

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 detection limit of the assay defined as the concentration of CA153 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 detection limit is typically less than 4 IU/ml.

14.4 Specificity
The specificity of the CA15-3 assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

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

15 References