MAGLUMI AFP (CLIA)

REF 130201002M

Σ 100

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EC REP

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CE

FOR PROFESSIONAL USE ONLY
Store at 2-8°C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

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SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in the European community
	Manufacturer
	Consult instructions for use
CONT	Contents of kit
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
8°C 2°C	Temperature limitation (store at 2-8°C)
Σ	Sufficient for
	Keep away from sunlight
	Keep upright for storage

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 1.25-1000.00IU/ml.
The test has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Including Maglumi 600,Maglumi 1000,Maglumi 1000 Plus, Maglumi 2000,Maglumi 2000 Plus,Maglumi 3000 and Maglumi 4000).

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 test; 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.
AFP is used to help detect and diagnose cancers of the liver, testes, and ovaries. It is often ordered to monitor people with chronic liver diseases such as cirrhosis or chronic hepatitis B because they have an increased lifetime risk of developing liver cancer. A doctor may order an AFP test, along with imaging studies, to try to detect liver cancer when it is in its earliest, and most treatable, stages.
If a patient has been diagnosed with hepatocellular carcinoma or another form of AFP-producing cancer, an AFP test may be ordered periodically to help monitor a patient's response to therapy and to monitor for cancer recurrence.
AFP is a protein produced primarily by fetal liver and the portion of a developing embryo that is similar to the yolk cavity in bird eggs (yolk sac tissues). AFP concentrations are typically elevated when a baby is born and then decline rapidly. In healthy children and non-pregnant adults, AFP is normally only detectable at very low levels. Liver damage and certain cancers can increase AFP concentrations significantly. AFP is produced whenever liver cells are regenerating. With chronic liver diseases, such as hepatitis and cirrhosis, AFP may be chronically elevated. Very high concentrations of AFP may be produced by certain tumors. This characteristic makes the AFP test useful as a tumor marker. Increased amounts of AFP are found in many people with a type of liver cancer called hepatocellular carcinoma. They are also found in some people with cancers of the testes and ovaries.

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 are mixed thoroughly with FITC Label and nano magnetic microbeads in a cuvette incubated at 37°C, then cycle washing for 1 time. Then add ABEI Label and incubated to form a sandwich, after sediment in a magnetic field, suck the supernatant then cycle

washing for the 2nd time. Subsequently, Starter1+2 substrates 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 samples.

KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN_3, coated with sheep anti- FITC polyclonal antibody.	2.5ml
Calibrator Low: bovine serum, 0.2%NaN_3.	2.5ml
Calibrator High: bovine serum, 0.2%NaN_3.	2.5ml
FITC Label: anti-AFP monoclonal antibody labeled FITC, containing BSA, 0.2%NaN_3.	12.5ml
ABEI Label: anti-AFP monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN_3.	22.5ml
Diluent: 0.9%NaCl	25ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml

Internal quality control is only applicable with MAGLUMI system. Instructions for use and target value refer to Quality Control Information date sheet. User needs to judge results with their own standards and knowledge.

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Please order accessories from SNIBE or our representative.

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 color of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.
- Keep upright for storage
- Keep away from sunlight

TRACEABILITY AND CALIBRATION

1)Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the W.H.O.1st International Standard AFP.

2) 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.

3) Frequency of Recalibration

- After each exchange of lots (Reagent Integral or Starter Reagents).
- Every 4 weeks and/or each time a new Integral is used (recommendation).
- After each servicing of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI.
- If controls are beyond the expected range.
- The room temperature has changed more than 5℃ (recommendation).

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect 5.0ml venous blood into Blood Collection Tube. Standing at room temperature，centrifuging, separating serum part.

The serum sample is stable for up to 12 hours at 2-8℃. More than 12 hours, please packed, -20 ℃ can be stored for 30 days.

Avoid repeated freezing and thawing, the serum sample can be only frozen and thawed two times. Stored samples should be thoroughly mixed prior to use (Vortex mixer).

If sediments appeared in the specimens，it should be centrifugate before analysis.

Please ask local representative of SNIBE for more details if you have any doubt.

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.

(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of

specimens should be avoided.

- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum from the serum separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 12 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping

Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

IVD

- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

- Results of the kits are only for clinical reference**.** For the patient's clinical diagnosis and treatment should be combined with its symptoms, signs, history, other laboratory tests and treatment reaction，and then take them into consideration compositely .
- It may have different results in using different manufacturers reagent for the same sample to detect tumor marker， because of the methodology ，specificity of the antibody and so on. To avoid the wrong medicine interpretation, in the process of monitoring tumor，the different reagent testing results should not be directly compared with each other. Suggest the laboratories give test reports to the clinical doctor indicating the reagent characteristics. When the reagent type changed in the series of monitoring, it should be has extra continuity testing and compare with the original reagent results parallelly to determine the baseline value again.
- 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，even they have passed the tests of HBs-Ag，HIV1/2-Ab，HCV-Ab and so on; these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.
- 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 auto-claved using an overkill approach. 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.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Blood borne Pathogens13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface; please pay attention to the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

40μl +100μl +20μl	Sample, calibrator FITC label Nano magnetic microbeads
10 min	Incubation
400μl	Cycle washing
+200μl	ABEI label
10 min	Incubation
400μl	Cycle washing
3 s	Measurement

DILUTION

Samples with concentrations above the measuring range can be diluted. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

Availability of sample dilution by analyzer please refers to the MAGLUMI analyzer user software program. Dilution settings please follow MALGUMI analyzer operating instructions.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the

range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

Patients with malignancies may exhibit AFP values within the normal range. AFP concentrations may be elevated in case of liver cirrhosis, hepatitis or tyrosinaemia. 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. AFP is not always a tumor marker. Because AFP is produced by the fetus, levels are normally higher in pregnant women and in their newborns. AFP can temporarily increase whenever the liver is injured and regenerating, and moderate elevations can be seen with a variety of conditions. Because of this, AFP testing can give some false positives. In addition, not every cancer will produce AFP, so a person could still have cancer even when the AFP is normal. For these reasons, the AFP test should not be used to screen the general population for cancer.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin<65mg/dl, haemoglobin<2.2g/dl，Triglycerides<1500mg/dL，RF<1500IU/ml.

3) HAMA

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

4) High-Dose Hook

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For the MAGLUMI AFP assay, no high dose hook effect was observed when samples containing up to 1,000,000IU/ml.

RESULTS

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 Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.
- Conversion factor: 1ng/ml=0.83 IU/ml.

2) Interpretation of Results

- Reference values< 6.05IU/ml
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(IU/ml)	SD(IU/ml)	CV%
Level 1	8.58	0.69	7.41
Level 2	63.76	5.06	7.23
Level 3	179.1	14.71	6.19

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(IU/ml)	SD(IU/ml)	CV%
Level 1	8.86	0.62	7.02
Level 2	67.69	4.63	6.84
Level 3	183.28	13.03	7.11

2) Analytical 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 1.25IU/ml.

3) Specificity

The specificity of the AFP assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross reactivity
CEA	200 IU/ml	0.625%
CA125	200 IU/ml	0.625%
CA153	200 IU/ml	0.625%

4) Recovery

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

Expected	Mean Measuring	Recovery
229.9 IU/ml	232.6 IU/ml	101%

5) Linearity

Use AFP calibrator to prepare the six-point standard curve, measuring all points' RLU except point A, and then do four-parameter linear fitting in double logarithm coordinate, the absolute linear correlation coefficient(r) should be bigger than 0.9900.

Calibrator Point	Concentration IU/ml	Absolute linear correlation coefficient (r)
A	0	
B	5.0	r=0.9960
C	20.0	
D	50.0	
E	200.0	
F	500.0	

6) Method comparison

A comparison of MAGLUMI AFP(y) with a commercially available AFP test (x) using clinical samples gave the following correlations (IU/ml):

Linear regression
y = 4.4997x-0.3044
r = 0.9896

Number of samples measured: 317
The sample concentrations were between 1.50 and 1245.82IU /ml.

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