Snibe Diagnostic

CE REF 130201002M: 100 tests
130601002M: 50 tests

MAGLUMI® AFP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of Alpha-Fetoprotein (AFP) in human serum using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8).

SUMMARY AND EXPLANATION OF THE TEST

AFP is a glycoprotein of 591 amino acids and AFP is a major plasma protein produced by the yolk sac and the liver during fetal development. It is thought to be the fetal form of serum albumin. AFP binds to copper, nickel, fatty acids and bilirubin and is found in monomeric, dimeric and trimeric forms[1-3].

Principal tumors that secrete AFP are endodermal sinus tumor (yolk sac carcinoma), neuroblastoma, hepatoblastoma, and hepatocellular carcinoma. In the context of evidence-based medicine, AFP is validated at the highest level as a tumor marker for use in patients with nonseminomatous germ cell tumors[4-5]. For hepatocellular carcinoma (HCC), AFP cannot be considered to be specifically diagnostic of HCC, levels of AFP may be elevated in serum from patients with chronic disease; for example, research has indicated that AFP is not useful for screening in patients suffering from cirrhosis or Hepatitis C and therefore elevated AFP in these patients may not be indicative, or be only suggestive, of HCC[6]. AFP is considered a useful marker for post-treatment monitoring of HCC patients (e.g. for treatment efficacy or tumor recurrence). The value of such tests may be improved by parallel monitoring of other markers[7-8]. Rare AFP-secreting tumor types include carcinoma in a mixed Müllerian tumor. The Sertoli-Leydig cell tumor, which itself is rare, rarely secretes AFP. In Wilms tumor AFP is rarely elevated, but when it is elevated it may serve as a marker of disease progression or recurrence[9-11]. Evaluation of serum AFP levels also could be applied to fetal screening, and abnormally elevated AFP in the serum of a pregnant woman can have one or more of these sources: a problem with the fetus, a problem with the placenta, a tumor or liver disease in the woman, and a normally elevated AFP in the fetus or woman (some people naturally have very high AFP)[12-14].

PRINCIPLE OF THE TEST

The AFP assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-AFP monoclonal antibody are mixed thoroughly and incubated, and then perform a wash cycle. Then add ABEI labeled with another anti-AFP monoclonal antibody, mix thoroughly and incubate to form a sandwich. After precipitation in a magnetic field, decant the supernatant, and then perform another wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of AFP present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130201002M)	50 tests (REF: 130601002M)
Magnetic Microbeads	Coated with anti- AFP monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing bovine serum and AFP antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing bovine serum and AFP antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
ABEI Label	Anti-AFP monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	12.5 mL	7.5 mL
Diluent	0.9%NaCl.	25.0 mL	15.0 mL
Internal Quality Control	Containing bovine serum and AFP antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representatives.

CALIBRATION

Traceability: This method has been standardized against the WHO 1st International Standard AFP.

Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve (10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.

Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).
- Every 4 weeks and/or each time a new reagent kit is used (recommended).
- After instrument service is required.

- If control results lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***AFP (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- 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 clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed once. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 7 days at 2-8°C.
- Specimens can be stored up to 3 months frozen at -20°C or colder. Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of AFP is15 µL.

WARNING AND PRECAUTIONS FOR USERS

- IVD
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. 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. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

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.

The automatic sample dilution is available after dilution settings are done in the MAGLUMI and Biolumi series Fully-auto chemiluminescence immunoassay analyzer. user software. Please refer to the corresponding Analyzer Operating Instructions.

High-Dose Hook

For the AFP assay, no high dose hook effect was observed when samples containing AFP up to 1,000,000 IU/mL.

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. 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. 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.

RESULTS

Calculation of Results

The analyzer automatically calculates the AFP concentration of each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are reported in the unit of IU/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Conversion factor: IU/mLx1.21=ng/mL.

Interpretation of Results

The expected range for the AFP assay was obtained by testing 267 apparently healthy individuals in China, and gave the following expected value: <6.05 IU/mL (95^{th} percentile).

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the AFP assay was determined as described in the CLSI EP5-A2. 3 human serum pools and 3 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean(IU/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(IU/mL)	%CV	SD(IU/mL)	%CV	SD(IU/mL)	%CV
Serum Pool 1	10.436	0.349	3.34	0.541	5.18	0.643	6.16
Serum Pool 2	97.533	2.950	3.03	2.438	2.50	3.827	3.92
Serum Pool 3	501.001	10.239	2.04	8.859	1.77	13.540	2.70
Control 1	8.506	0.369	4.34	0.437	5.14	0.571	6.71
Control 2	64.488	2.216	3.44	1.567	2.43	2.714	4.21
Control 3	180.010	4.846	2.69	4.396	2.44	6.543	3.63

Limit of Blank (LoB)

The LoB for the AFP assay is 0.5 IU/mL.

Limit of Detection (LoD)

The LoD for the AFP assay is 0.75 IU/mL.

Measuring Range

0.5-1000 IU/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.5 IU/mL. Values above the measuring range are reported as >1000 IU/mL.

Linearity

The assay is linear between 0.75 IU/mL and 1000 IU/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a serum sample containing AFP 1100 IU/mL with a serum sample depleted of AFP (0.0 IU/mL).The mean sample recovery ranged between 90% to 110%.

Method Comparison

A total of 160 samples in the range of 0.570 and 964.322 IU/mL were tested by the AFP assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=0.950x +0.245, r2=0.968.

Analytical Specificity

The specificity of the assay was obtained by adding CEA (200 IU/mL), CA 125 (200 U/mL) and CA 153 (200 U/mL) to serum samples. No interference was found.

Endogenous Interference

Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 66 mg/dL
- Hemoglobin 2200 mg/dL
- Triglyceride 1500 mg/dL
- RF 1500 IU/mL

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code