Snibe Diagnostic

CE 0123 REF 130201033M:100 tests/kit
130601033M: 50 tests/kit
130701033M: 30 tests/kit

MAGLUMI® AFP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of AFP in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer and Biolumi series Integrated System, and the assay is used for an aid in the diagnosis of hepatocellular carcinoma (HCC).

SUMMARY

Alpha-fetoprotein (AFP) is a glycoprotein that is produced by the fetal liver and yolk sac during the first trimester of pregnancy[1]. Peak levels are noted during week 12 to 14 of gestation and then steadily decline so that 1 year after birth[2].

AFP has long been recognized as a tumor marker for HCC, and has played a prominent role in the diagnosis of HCC according to previous guidelines[3]. The test for AFP is the first serologic assay used for the detection and clinical follow-up of patients with HCC[1]. Clinical studies have shown a potential relationship between AFP level and progression of HCC, and AFP may be used as a marker for monitoring treatment response in HCC patients[4]. Serum AFP measurement is recommended for HCC surveillance by the Asian Pacific Association for the Study of the Liver (APASL) and the National Comprehensive Cancer Network (NCCN)[5,6]. In Japan, three tumor markers (AFP, PIVKA-II, AFP-L3) are covered by the national health insurance in clinical settings for HCC surveillance[7]. In a study by Rathmell et al, elevated AFP and/or HCG was the initial indicator of disease recurrence in 55% of patients with either nonseminoma germ cell tumors (NSCGTs) or seminoma[2]. Elevated serum AFP is associated with nonseminomatous germ cell tumors, particularly embryonal or yolk sac carcinomas, and may be seen at any disease stage[8].

Besides liver cancer, malignant tumors from stomach, pancreas, and reproductive system are often accompanied by a small amount of increased AFP[9]. Elevations in serum AFP levels can be seen in patients with acute hepatitis, chronic liver diseases, cirrhosis, colitis, ataxia telangiectasia, and fetal disorders, and at the onset of normal pregnancy with steady decline as the gestation progresses[10].

TEST PRINCIPLE

Sandwich chemiluminescence immunoassay.

The sample, buffer, magnetic microbeads coated with anti-AFP monoclonal antibody are mixed thoroughly, incubating and performing a wash cycle after a precipitation in a magnetic field. ABEI labeled with another anti-AFP monoclonal antibody are then added, reacting to form sandwich complexes and incubating. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. 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.

REAGENTS

Kit Contents

Component	Description	100 tests/kit	50 tests/kit	30 tests/kit
Magnetic Microbeads	Magnetic microbeads coated with anti-AFP monoclonal antibody (~6.00 μg/mL) in PBS buffer, NaN_3 (<0.1%).	2.5 mL	1.5 mL	1.0 mL
Calibrator Low	A low concentration of AFP antigen in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Calibrator High	A high concentration of AFP antigen in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Buffer	Tris-HCl buffer, NaN_3 (<0.1%).	13.5 mL	7.5 mL	4.8 mL
ABEI Label	ABEI labeled with anti-AFP monoclonal antibody (~0.056 μg/mL) in Tris-HCl buffer, NaN_3 (<0.1%).	13.5 mL	7.5 mL	4.8 mL
Diluent	0.9% NaCl.	5.0 mL	5.0 mL	3.0 mL
Control 1	A low concentration of AFP antigen (10.0 IU/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
Control 2	A high concentration of AFP antigen (100 IU/mL) in PBS buffer, NaN_3 (<0.1%).	1.0 mL	1.0 mL	1.0 mL
All reagents are provided ready-to-use.				

The control barcode labels are provided.

Warnings and Precautions

- For *in vitro* diagnostic use.
- For professional use only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples, reagents, and controls, and should comply with local operating requirements for the assay.
- A skillful technique and strict adherence to the package insert are necessary to obtain reliable results.
- Do not use kit beyond the expiration date indicated on the label.
- Do not interchange reagent components from different reagents or lots.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- All waste associated with biological samples, biological reagents and disposable materials used for the assay should be considered potentially infectious and should be disposed of in accordance with local guidelines.
- This product contains sodium azide. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. Immediately after disposal, flush with a large volume of water to prevent azide build-up. For additional information, see Safety Data Sheets available for professional user on request.

Note: If any serious incident has occurred in relation to the device, please report to Shenzhen New Industries Biomedical Engineering Co., Ltd. (Snibe) or our authorized representative and the competent authority of the Member State in which you are established.

Reagent Handling

- To avoid contamination, wear clean gloves when operating with a reagent kit and sample. When handling reagent kit, replace the gloves that have been in contact with samples, since introduction of samples will result in unreliable results.
- Do not use kit in malfunction conditions; e.g., the kit leaking at the sealing film or elsewhere, obviously turbid or precipitation is found in reagents (except for Magnetic Microbeads) or control value is out of the specified range repeatedly. When kit in malfunction conditions, please contact Snibe or our authorized distributor.
- To avoid evaporation of the liquid in the opened reagent kits in refrigerator, it is recommended that the opened reagent kits to be sealed with reagent seals contained within the packaging. The reagent seals are single use, and if more seals are needed, please contact Snibe or our authorized distributor.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts and have no effect on assay efficacy.
- Use always the same analyzer for an opened reagent integral.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- For further information about the reagent handing during system operation, please refer to Analyzer Operating Instructions.

Storage and Stability

- Do not freeze the integral reagents.
- Store the reagent kit upright to ensure complete availability of the magnetic microbeads.
- Protect from direct sunlight.

Stability of the Reagents	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
On-board	4 weeks

Stability of Controls	
Unopened at 2-8°C	until the stated expiration date
Opened at 2-8°C	6 weeks
Opened at 15-25°C	6 hours
Frozen at -20°C	3 months
Frozen and thawed cycles	no more than 3 times

SPECIMEN COLLECTION AND PREPARATION

Specimen Types

Only the specimens listed below were tested and found acceptable.

Specimen Types	Collection Tubes
Serum	Tubes without additive/accessory, or tubes containing clot activator or clot activator with gel
Plasma	K2-EDTA

- The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. Follow tube manufacturers' instructions carefully when using collection tubes.

Specimen Conditions

- Do not use heat-inactivated samples or grossly hemolyzed/hyperlipidaemia specimens and specimens with obvious microbial contamination.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some serum specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the serum 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.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Inspect all specimens for foam. Remove foam with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.
- Frozen specimens must be completely thawed before mixing. Mix thawed specimens thoroughly by low speed vortexing or by gently inverting. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous. If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Specimens should be free of fibrin, red blood cells, or other particulate matter. Such specimens may give reliable results and must be centrifuged prior to testing. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material..
- The sample volume required for a single determination of this assay is 15 μL.

Specimen Storage

Specimens removed from the separator, red blood cells or clot may be stored up to 8 hours at 15-25°C, or 7 days at 2-8°C, or 3 months frozen at -20°C or colder. Frozen specimens subjected to up to 3 freeze/thaw cycles have been evaluated.

Specimen Shipping

- Package and label specimens in compliance with applicable local regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

Specimen Dilution

- Samples, AFP concentrations above the analytical measuring interval, can be diluted with Diluent either automated dilution protocol or manual dilution procedure. The recommended dilution ratio is 1:50 (sample volume:total volume). The concentration of the diluted sample must be >20 IU/mL.
- For manual dilution, multiply the result by the dilution factor. For dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.

PROCEDURE

Materials Provided

AFP (CLIA) assay, control barcode labels.

Materials Required (But Not Provided)

- General laboratory equipment.
- Fully-auto chemiluminescence immunoassay analyzer Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3, MAGLUMI X6, or Integrated System Biolumi 8000 and Biolumi CX8.
- Additional accessories of test required for the above analyzers include Reaction Module, Starter 1+2, Wash Concentrate, Light Check, Tip, and Reaction Cup. Specific accessories and accessories' specification for each model refer to corresponding Analyzer Operating Instructions.
- Please use accessories specified by Snibe to ensure the reliability of the test results.

Assay Procedure

Preparation of the Reagent

- Take the reagent kit out of the box and visually inspect the integral vials for leaking at the sealing film or elsewhere. If there is no leakage, please tear off the sealing film carefully.
- Open the reagent area door; hold the reagent handle to get the RFID label close to the RFID reader (for about 2s); the buzzer will beep; one beep sound indicates successful sensing.
- Keeping the reagent straight insert to the bottom along the blank reagent track.
- Observe whether the reagent information is displayed successfully in the software interface, otherwise repeat the above two steps.
- 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.

Assay Calibration

- Select the assay to be calibrated and execute calibration operation in reagent area interface. For specific information on ordering calibrations, refer to the calibration section of Analyzer Operating Instructions.
- Execute recalibration according to the calibration interval required in this package insert.

Quality Control

- When new lot used, check or edit the quality control information.
- Scan the control barcode, choose corresponding quality control information and execute testing. For specific information on ordering quality controls, refer to the quality control section of the Analyzer Operating Instructions.

Sample Testing

- After successfully loading the sample, select the sample in interface and edit the assay for the sample to be tested and execute testing. For specific information on ordering patient specimens, refer to the sample ordering section of the Analyzer Operating Instructions.

To ensure proper test performance, strictly adhere to Analyzer Operating Instructions.

Calibration

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

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the master curve.

Recalibration is recommended as follows:

- Whenever a new lot of Reagent or Starter 1+2 is used.
- Every 28 days.
- The analyzer has been serviced.
- Control values lie outside the specified range.

Quality Control

Controls are recommended for the determination of quality control requirements for this assay and should be run in singlicate to monitor the assay performance. Refer to published guidelines for general quality control recommendations, for example Clinical and Laboratory Standards Institute (CLSI) Guideline C24 or other published guidelines[11].

Quality control is recommended once per day of use, or in accordance with local regulations or accreditation requirements and your laboratory's quality control procedures, quality control could be performed by running the AFP assay:

- Whenever the kit is calibrated.
- Whenever a new lot of Starter 1+2 or Wash Concentrate is used.

Controls are only applicable with MAGLUMI and Biolumi system and only used matching with the same top seven LOT numbers of corresponding reagents. For each target value and range refer to the label.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

Control values must lie within the specified range, whenever one of the controls lies outside the specified range, calibration should be repeated and controls retested. If control values lie repeatedly outside the predefined ranges after successful calibration, patient results must not be reported and 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 package insert.
- If necessary, contact Snibe or our authorized distributors for assistance.

If the controls in kit are not enough for use, please order AFP (CLIA) Controls (REF: 160201220MT) from Snibe or our authorized distributors for more.

RESULTS

Calculation

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 Analyzer Operating Instructions.

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

Interpretation of Results

The expected range for the AFP assay was obtained by testing 525 apparently healthy individuals in China, gave the following expected value:

≤6.05 IU/mL (95th percentile).

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

LIMITATIONS

- Results should be used in conjunction with patient's medical history, clinical examination and other findings.
- If the AFP results are inconsistent with clinical evidence, additional testing is needed to confirm the result.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies[12,13]. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed[14].
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- The assay is not suitable for screening of the general population. The intended testing population are individuals suspected or diagnosed with tumors.

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

Precision

Precision was determined using the assay, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): duplicates at two independent runs per day for 5 days at three different sites using three lots of reagent kits (n =180). The following results were obtained:

Sample	Mean (IU/mL) (n=180)	Within-Run		Between-Run		Reproducibility	
		SD (IU/mL)	%CV	SD (IU/mL)	%CV	SD (IU/mL)	%CV
Serum Pool 1	5.956	0.237	3.98	0.154	2.59	0.339	5.69
Serum Pool 2	99.779	3.401	3.41	1.880	1.88	5.706	5.72
Serum Pool 3	595.642	18.330	3.08	11.275	1.89	22.708	3.81
Plasma Pool 1	5.955	0.258	4.33	0.055	0.92	0.349	5.86
Plasma Pool 2	97.510	3.437	3.52	0.940	0.96	4.094	4.20
Plasma Pool 3	592.146	16.597	2.80	14.533	2.45	27.361	4.62
Control 1	9.990	0.415	4.15	0.197	1.97	0.543	5.44
Control 2	101.943	3.213	3.15	2.165	2.12	5.366	5.26

Based on internal testing on the MAGLUMI and Biolumi systems, the overall repeatability (% CV) is estimated to be ≤ 5% for samples tested, the overall reproducibility (%CV) is estimated to be ≤ 10%. Performance of the assay at individual laboratories may vary.

Trueness

Trueness (bias) was determined using the assay and five samples containing different concentrations of WHO 1st International Standard AFP in a protocol (EP15-A3) of the CLSI (Clinical and Laboratory Standards Institute). The observed mean is within the verification interval.

Linear Range

0.800-1000 IU/mL (defined by the Limit of Quantitation and the maximum of the master curve).

Reportable Interval

0.700-50000 IU/mL (defined by the Limit of Detection and the maximum of the master curve×Recommended Dilution Ratio).

Analytical Sensitivity

Limit of Blank (LoB) =0.500 IU/mL.

Limit of Detection (LoD) =0.700 IU/mL.

Limit of Quantitation (LoQ) =0.800 IU/mL.

Analytical Specificity

Interference

Interference was determined using the assay, three samples containing different concentrations of analyte were spiked with potential endogenous and exogenous interferents in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Interference	No interference up to	Interference	No interference up to
Bilirubin	66 mg/dL	Cisplatin	165 μg/mL
Hemoglobin	2200 mg/dL	Methotrexate	450 μg/mL
Intralipid	1500 mg/dL	5-Fluorouracil	360 μg/mL
HAMA	40 ng/mL	Paclitaxel	67 μg/mL
Rheumatoid factor	1500 IU/mL	Vinblastine sulfate	1.5 μg/mL
ANA	6(S/CO) strong positive	Doxorubicin hydrochloride	50 μg/mL
Cyclophosphamide monohydrate	500 μg/mL	Bleomycin	1300 μg/mL
Carboplatin	500 μg/mL		

Cross-Reactivity

Cross-reactivity was determined using the assay, three samples containing different concentrations of analyte were spiked with potential cross-reactants in a protocol (EP7-A2) of the CLSI. The measurement deviation of the interference substance is within ±10%. The following results were obtained:

Cross-reactant	No interference up to	Cross-reactant	No interference up to
Prolactin	10000 μIU/mL	Transferrin	4 mg/mL
hCG	1000 IU/mL	Ferritin	10000 ng/mL

High-Dose Hook

No high-dose hook effect was seen for AFP concentrations up to 1000000 IU/mL.

Method Comparison

A comparison of the AFP assay with a commercially available immunoassay, gave the following correlations (IU/mL):

Number of samples measured: 314

Passing-Bablok: $y=1.0241x-0.0534$, $\tau=0.961$.

The clinical specimen concentrations were between 0.905 and 996.7 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<n> tests		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit component
REF	Catalogue number	LOT	Batch code
CE 0123	CE marking with notified body ID number		

Summary of safety and performance is available at Eudamed.

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