Alpha-fetoprotein (CLIA) PACKAGE INSERT

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INTENDED USE

Alpha-fetoprotein (CLIA) is a chemiluminescence immunoassay test for the quantitative measurement of alpha-fetoprotein (AFP) in human serum or plasma (Heparin lithium, EDTA-K_2 or EDTA-K_3). which is intended as an aid to assess the auxiliary diagnosis, curative effect and prognosis observation of primary liver cancer. The test must be performed on the ACCRE series analyzers (including ACCRE 6 and ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120 and all other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

SUMMARY

Alpha-fetoprotein (AFP) is a single chain glycoprotein and the molecular weight of AFP is 70 KDa. The biological structure of AFP is highly similar to albumin. AFP is the major composition of serum protein in fetal period. AFP was generated by the fetal saccus vitellinus, undifferential hepatic cell and gastrointestinal tract[1,2]. AFP can be detected after fertilization, and the concentration reach to the peak at 12-15 weeks of pregnancy, then gradually drop. The concentration of AFP will drop to the level of regular adult after 2 years old [3,4].

Elevation of AFP can be the indication of pregnancy or sever disease such as primary liver cancer, hepatocirrhosis, acute viral hepatitis or non-spermatogenic testicular carcinoma [5,6,7,8]. Significant evaluation of AFP was often indicate the primary liver cancer, but the concentration of AFP did not shows relevant correlation with the size of tumor, the transfer of tumor or the progression of tumor.

AFP can be used for the screen of trisomy 21 syndrome in combination with β-HCG. The concentration of AFP will drop while the concentration of β-HCG in trisomy 21 syndrome patient.

The AFP assay was recommended for the monitoring of above related disease, not recommend for the screen and diagnostic of malignant tumor.

ASSAY PRINCIPLES

The Alpha-fetoprotein (CLIA) hereinafter also referred to as the 'AFP' is a two-sites immunoassay sandwich method with enzyme catalytic chemiluminescence detection (CLIA). The principle is described as follow:

The sample is manually pipetted into the sample well of reagent cartridge. The sample and the mouse anti-AFP monoclonal antibody labeled alkaline phosphatase are added to the magnetic beads coated with the mouse anti-AFP monoclonal antibody, and after mixing and incubation, the antigen in the sample and the antibody on the magnetic beads and the antibody labeled alkaline phosphatase binds to form a complex.

After the reaction was completed, the magnetic field adsorbed the magnetic beads and the unbound material was removed by three washings. After the reaction substrate was added, the luminescence intensity was measured by a photomultiplier tube. The concentration of the substance to be detected in the sample is proportional to the intensity of the luminescence. The amount of analyte in the sample is determined by a calibration curve.

CONTENT OF THE KIT

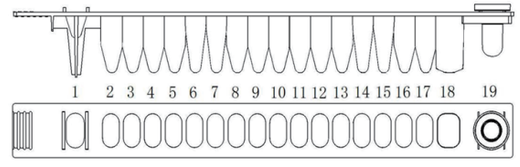
Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls, 60 tests/kit without quality controls, 36 tests/kit without quality controls

The calibration kit has been traced to the national standard 150542 for alpha-fetoprotein immunoassay. The exact calibrator concentration values are detailed in the kit calibration cards.

Contents	Quantities				Notice
	REF W60A	REF W60C	REF W76A	REF W76C	
AFP reagent cartridge	60 strips	36 strips	60 strips	36 strips	Ready to Use
AFP Calibrator 1 (CAL1)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	MOPS buffer,50mM; ProClin300,0.48g/L; BND,0.2g/L; AFP recombinant protein, 5.0 IU/mL
AFP Calibrator 2 (CAL2)	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	1.0 mL x 1 vial	MOPS buffer,50mM; ProClin300,0.48g/L; BND,0.2g/L; AFP recombinant protein, 250 IU/mL
AFP Quality Control Level 1 (QC1)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	MOPS buffer,50mM; ProClin300,0.48g/L; BND,0.2g/L; AFP recombinant protein, 7IU/mL Only for the configuration with Quality Controls
AFP Quality Control Level 2(QC2)	1.0 mL x 1 vial	1.0 mL x 1 vial	/	/	MOPS buffer, 50mM ; ProClin300,0.48g/L; BND,0.2g/L; AFP recombinant protein, 100IU/mL Only for the configuration with Quality Controls
Instruction for Use	1	1	1	1	N/A
Reagent Registration Card	1	1	1	1	N/A
Calibrator Barcode Sheet	1	1	1	1	N/A
Quality Control Sheet	1	1	/	/	Only for the configuration with Quality Controls

The Reagents Cartridge

The reagent strip consists of 19 wells. The 1st well is the sample well and the last well 19th is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a label, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagent components needed for the assay are ready-to-use and pre-dispensed in the cartridges. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
5	Sample Diluent	Tris buffer,50mmol/L; ProClin300, 0.48g/L; BND, 0.2g/L	160μL
7	Magnetic Particles	Anti-AFP antibody(mouse) coated magnetic particles,0.2g/L; Tris buffer,50mmol/L; ProClin300,0.48g/L; BND,0.2g/L	50μL
9	Enzyme Label	ALP (alkaline phosphatase) labeled anti-AFP antibody (mouse),2.0 mg/L; MES Buffer, 50mmol/L ; ProClin300,0.48g/L; BND,0.2g/L	50μL
10, 11, 12	Washing Solutions	Tris buffer,50mmol/L; ProClin300,0.48g/L; BND,0.2g/L	350μL
16	Substrate	(4-chlorophenyl sulfhydryl) (10-methyl-9, 10-dihydroacridine methylene) disodium phosphate salt	100μL

Note: Well 1, 2, 3, 4, 6, 8, 13, 14, 15, 17, 18 are empty.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Sample diluent (manufactured by Tisenc).
- Pipette with disposable tip to dispense 200 μL.
- Powderless and disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adhere to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Do not mix reagents (or disposables) from different lots.
- 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.
- Use powder less gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

STORAGE

- Store the Alpha-fetoprotein (CLIA) kits **upright** at 2-8°C, kits can be stored for 18 months from manufactured date. After the reagent cartridge is on-board in the ACCRE analyzer, the testing should be finished within 2 hours.
- Store the calibrators and quality controls at 2-8°C till expiry date. When opened, the calibrators and/or controls will be stable for 30 days at 2-8°C and longer time at -20°C or below -20°C for 90 days. Calibrators and quality controls can only be frozen and thawed once.
- Keep away from sunlight.
- Do not freeze reagents cartridge.

SAMPLES

The sample volume for Alpha-fetoprotein (CLIA) assay is 40μL, please note that at least 70μL of sample has to be pipetted manually into the reagent catrridge.

Sample type and collection

Human serum or plasma (Heparin lithium,EDTA-K_2 or EDTA-K_3) are recommended. Types of tubes:

- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with Heparin lithium,
- Plastic tube with EDTA-K_2.
- Plastic tube with EDTA-K_3.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.

Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.

It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation

The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.

For use of the sample tubes, follow the tube manufacturer's instructions for use.

The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.

Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible

to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.

Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.

If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.

Severe hemolysis samples (hemoglobin >500mg/dL) or heat-inactivated samples are not recommended for applying on the reagents.

Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.

Repeated freezing and thawing is not allowed.

Mix using with a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability

Samples (serum and plasma) can be stored at room temperature (15-30°C） for up to 4 hours or at 2~8°C for up to 7 days; if longer storage is required, freeze the serum or plasma at -20°C or below -20°C for up to 3 months.

INSTRUCTIONS FOR USE

For complete instructions, see the Instrument User Manual.

When using the assay for the first time.

Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.

When opening a new lot of reagents: Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer. Calibration is necessary under the following conditions:

(1) New reagent lot number applied;

(2) Same lot of reagents used more than 4 weeks;

(3) Quality control out of control;
(4) When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1. Remove the kit from storage at 2-8°C and take out the required number of reagent cartridges. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.
2. Use each assay strips for each sample, calibrator or control respectively.
3. Place the reagent cartridges into the reagent rack.
4. Manually pipette the Sample, Calibrator or Control into the sample well of each reagent cartridge.

Please note that at least 70μL sample has to be pipetted manually into the reagent cartridge and 40μL sample is needed for each test.

** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent（if applicable) DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of the ACCRE analyzer.
6. Place the assay tips into the reagent chamber of the ACCRE analyzer.
7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.
8. Alpha-fetoprotein (CLIA) assay will be completed within approximately 25 minutes. Assays will be performed automatically by the ACCRE analyzer.
9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 1000 IU/mL can be diluted with negative serum or plasma manually or sample diluent manually. The recommended dilution is 1:10. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples.

Quality Control

Quality control should be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

RESULTS AND INTERPRETATION

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 2-point calibration, and calculated according to a predefined mathematical model, the results expressed in **IU /mL**.
Conversion factors: IU/mL x 1.21 = ng/mL ; ng/mL x 0.83 = IU/mL.

Interpretation of Results

Results of AFP assays are determined by testing specimens from a study in clinical centers with group of totally 294 healthy adult individuals in China (153 males and 141 females). The 97.5th percentile value is 5.987IU /mL

** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*

LIMITATIONS

1. For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
2. Please note that heterophile antibody in human serum or plasma can interfere the test result.
3. High concentration of HAMA can also cause a false positive or false negative result.
4. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
5. The detection range of the kit is 0.5IU/mL to 1000IU/mL. Samples with AFP concentration below the upper limit can be quantified, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <0.5IU/mL; if the concentration is above the upper detection limit, the results are reported as >1000IU/mL. The product supports sample dilution of 1:10 and can be diluted with negative serum or plasma manually or sample diluent.

PERFORMANCE CHARACTERISTICS

Lower limit of measurement

LOD (Limit of Detection) = 0.5IU/mL
LOD was determined in accordance with the CLSI® (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).
The measurement range of the Alpha-fetoprotein (CLIA) assay is: 0.5IU/mL~1000IU/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Alpha-fetoprotein (CLIA) assay is linear between 0.5IU/mL to 1000IU/mL. The absolute linear correlation coefficient(r) should be bigger than 0.9900.

Precision

The precision study was performed according to CLSI® EP05-A3 recommendations.
The Within-lot precision CV% is less than 8%.
The between-lot precision CV% is less than 15%.

Interfering Substances

Potential interference substances were studied according to CLSI® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤500mg/dL
Bilirubin	≤20mg/dL
Triglyceride	≤1500mg/dL
Rheumatoid factor	≤1500IU/mL

Accuracy

The sample with the correctness control product with traceability is used for the detection, and the relative deviation between the detection result and the calibration concentration is within ±10%.

WASTE DISPOSAL

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
IVD	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit	LOT	Batch code	REF	Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
EU REP	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use	BIO	Contains biological material of animal origin
	Biological risks		Caution	CE	CE marking
	Upright		Fragile, handle with care		Do not roll
CONTROL	Control				

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