Hepa AFP-IC
ELISA Kit for the detection of Alpha-Fetoprotein (AFP) Immune Complexes in Hepatocellular Carcinoma (HCC)

INTENDED USE
Hepa AFP-IC is an enzyme linked immunosorbent assay (ELISA) for the quantitative measurement of alpha-fetoprotein (AFP) immune complexes (AFP-IgM) in serum samples.

SUMMARY AND EXPLANATION OF THE TEST
Hepa AFP-IC belongs to a novel generation of in vitro diagnostic methods based on the detection of AFP as circulating Immune Complexes (IC). Hepa AFP-IC is a highly specific and sensitive ELISA assay for HCC detection designed to measure AFP-IgM in patient sera. The amount of AFP-IgM is expressed in Arbitrary Units (AU/mL), using a specific calibrator as reference. Studies have demonstrated that the measurement of AFP-IgM levels in HCC patients detects liver cancer with higher sensitivity with respect to AFP, the well-established serum HCC biomarker, without compromising specificity (1-3). Furthermore, the occurrence of the free and IgM-complexed form of the circulating AFP does not overlap, indicating that AFP-IgM is complementary to AFP and the use of both markers can increase sensitivity to over 80% (1-4).

PRINCIPLE OF THE TEST
Standard Calibrators and specimens are simultaneously incubated with anti-AFP antibodies coated to the wells of a microtiter plate. The immune complexes AFP-IgM are detected by the addition of an enzyme conjugated secondary antibody and an enzyme substrate (TMB). The developed color is proportional to the amount of the analyte in the sample.

REAGENTS AND MATERIALS PROVIDED
- XG005-PL: 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-AFP.
- XG005-Calibrator*: Two vials of calibrator lyophilized from PBS. White powder. Exact concentration on label. Totally soluble.
- XG-EA: 200 µL of Enzyme-conjugated goat anti-human IgM secondary antibody (Green cap) 100-fold concentrate in stabilizer solution. The solution contains Proclin as preservative.
- XG-GH3: 11 mL of TMB (3,3’,5,5’-Tetramethylbenzidine) chromogen solution ready to use.
- XG-ST3: 11 mL of 1N HCl Stop solution ready to use.
- XG-DBS: 10 mL of concentrated Dilution Buffer solution 5X. Once diluted, the working solution contains 1% BSA and 0.05% Tween 20 in PBS. The solution contains Proclin as preservative.
- XG-WB2: Two tablets of lyophilized Washing Buffer. Once dissolved, the working solution contains 0.05% Tween 20 in PBS. Totally soluble.
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MATERIAL AND EQUIPMENT REQUIRED
- Precision pipettes with disposable tips
- Microplate washer
- Microplate readers with a 450 ± 20 nm filter
- Distilled or deionized water

STORAGE CONDITIONS
- Storage at 4°C: XG005-PL, XG-CH3, XG-ST3, XG-DB5, XG-WB2
- Storage at -20°C: XG-EA, XG005-Calibrator®.

WARNING - POTENTIAL BIOHAZARDOUS MATERIALS
The standard calibrator XG005-Calibrator is of human origin. The reference material was tested using an approved method of evaluation for the presence of the antibodies to HIV, antibodies to the hepatitis C virus and hepatitis B surface antigens, and found to be negative. Since no test method can offer complete assurance that HIV, hepatitis B virus, hepatitis C virus, or other infectious agents are absent, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens (4). Biosafety Level 2 (5) or other appropriate biosafety practices (6, 7) should be used for materials that contain or are suspected of containing infectious agents.

SPECIMEN COLLECTION AND PREPARATION
The use of serum samples are recommended for the Hepa AFP-IC assay. Serum specimens should be collected aseptically, avoiding hemolysis if possible.
3. Remove excess microtiter plate strips from the frame and store in the re-sealable foil bag with the desiccant provided.
4. Wash the microtiter plate strips three times with XG-WB2 washing buffer (300 µL/well).
5. Dispense 100 µL/well of standard calibrators (in duplicate) starting from the reconstituted solution and performing in-plate 2-fold serial dilutions in order to obtain a seven-point calibration curve. Use XG-DB5 dilution buffer as diluent (videoclip available at http://www.xeptagen.com/ellisains).

PROCEDURAL NOTES
- Allow samples and reagents to reach room temperature prior to testing. Do not use water baths to thaw samples or reagents.
- Mix samples and all reagents thoroughly before use.
- Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.
- Avoid handling the tops of the wells both before and after filling.
- Standards and samples should be assayed in duplicate.
- Run a separate standard curve for each assay.
- Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.
- Perform incubations in a sealed box containing a wet paper towel in order to prevent evaporation.

REAGENTS PREPARATION
- Reconstitute XG005-Calibrator with 440 µL of deionized water for each calibration vial.
- Prepare the required amount of XG-DB5 dilution buffer by diluting 5-fold the concentrated solution in deionized water. If crystals appear upon refrigeration, warm the bottle to 37°C with mixing to dissolve.
- Reconstitute 1 tablet of XG-WB2 washing buffer in 500 mL of deionized water. Prepare 1 liter with two tablets of XG-WB2 washing buffer for the entire plate.
- Prepare the required amount of XG-EA enzyme-conjugated secondary antibody solution diluting 100-fold in reconstituted XG-DB5 dilution buffer.

ASSAY PROTOCOL
1. Prepare assay reagents as described above.
2. Set up the microtiter plate with sufficient wells to enable the running of all required standards and samples.
3. Add 100 µL/well of a hundred fold (1:100) diluted sample (in duplicate). Use XG-DB5 dilution buffer as diluent.
4. Incubate 1 hour at room temperature.
5. Wash six times with XG-WB2 washing buffer (300 µL/well).
6. Incubate 1 hour at room temperature.
7. Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution.
8. Wash six times with XG-WB2 washing buffer (300 µL/well).
9. Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution.
10. Wash six times with XG-WB2 washing buffer (300 µL/well).
11. Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution.
12. Wash six times with XG-WB2 washing buffer (300 µL/well).
13. Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution.

PROGRESSING OF THE RESULTS
Average the duplicate readings for each standard calibrator and sample and subtract the zero standard optical density. The standard calibrators may be used to construct two distinct standard curves with values reported in AU/mL. Plot on a semi-logarithmic graph the OD readings corresponding to the reconstituted standard calibrator and those corresponding to 1.2, 1.4 and 1.8 dilutions. Plot on a linear graph the OD readings corresponding to the AFP-IgM titers obtained diluting 1/8 to 1/64 the reconstituted standard calibrator, and the point (0.0). Data deduction may be performed through computer methods using curve fitting routines or may also be manually deduced on paper.

QUALITY CONTROL
The intra- and inter-assay coefficients of variation were determined on 4 typical standard curves and the results were less than 10%. For optimal performance, the absorbance of the zero standard should be < 0.2 OD450.

It is recommended that each laboratory assays appropriate quality control samples in each run to ensure that all reagents and procedures are correct.

INTERPRETATION
The AFP-IgM cut-off value was 120 AU/mL for differentiating HCC from non-malignant chronic liver diseases (2,3).

SPECIFIC PERFORMANCE CHARACTERISTICS
The linear range of the assay is 0-30 AU/mL. The sample with values above 30 AU/mL should be further diluted and re-measured.

REFERENCES