



PRODUCT DATA SHEET



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Hepa AFP-IC

XG005

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

Rev. 04/2010

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-CH3: 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-DB5: 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 diluted, the working solution contains 0.05% Tween 20 in PBS. Totally soluble.

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[§].

Avoid repeated freeze and thaw cycles

(*) Must be used within one month of reconstitution

(§) Must be reconstituted just before the use

EXPIRATION DATE

Expiration date printed on the kit indicates limits of stability.

WARNINGS - 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.



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Specimens should be stored at 2-8°C if the assay will be performed within 24 hours after collection. Specimens should be stored frozen if testing will occur after 24 hours.

If frozen, specimens should be mixed thoroughly after thawing to ensure consistency in the results. Avoid repeated freezing and thawing. Specimens showing particulate matter, erythrocytes, or turbidity must be clarified by centrifugation before testing.

INSTRUCTIONS FOR USE

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 calibrator 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. 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/elisains>). For exact concentration of the reconstituted calibrator please refer to the concentration value (AU/mL) indicated on the XG005-Calibrator vial. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank, in duplicate.
6. Dispense 100 µL/well of a hundred fold (1:100) diluted sample (in duplicate). Use XG-DB5 dilution buffer as diluent.
7. Incubate 1 hour at room temperature.
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. Incubate 1 hour at room temperature.
11. Wash six times with XG-WB2 washing buffer (300 µL/well).
12. Apply 100 µL/well of XG-CH3 chromogen solution.
13. Allow color to develop for 10-15 min at room temperature in the dark then apply 100 µL/well of XG-ST3 Stop Solution and measure OD values of each well using an ELISA plate reader equipped with a 450 nm filter. Stopped reaction should be read within 1 hour.
14. Elaborate OD values with Xerepro software (<http://www.xeptagen.com/software>) or plot the standard curve ΔOD values as described in the next section: Processing of the results.

PROCESSING 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.

The immune complexes concentration in the biological sample can be calculated directly from the appropriate



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standard curve, depending on the sample absorbance value, by interpolation. The value obtained has to be multiplied by the dilution factor of the sample. Samples with OD values exceeding the upper calibration limit should be further diluted and re-measured.

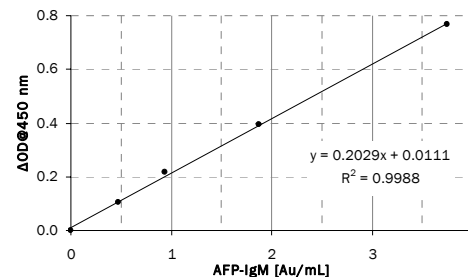


FIG. 1A: range of linearity (0 to 3.75 AU/mL) of a typical linear standard curve for AFP-IgM after 15 minutes of substrate incubation at room temperature and addition of stop solution

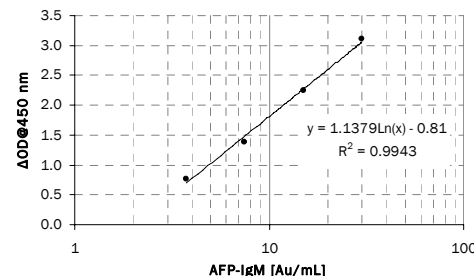


FIG. 1B: range of linearity (from 3.75 to 30.0 AU/mL) of a typical semi-logarithmic standard curve for AFP-IgM after 15 minutes of substrate incubation at room temperature and addition of stop solution

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.

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