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Colon-IC

XG006

ELISA Kit for the detection of Carcinoembryonic Antigen (CEA) Immune Complexes in Colorectal Carcinoma (CRC)

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INTENDED USE

Colon-IC is an enzyme linked immunosorbent assay (ELISA) for the quantitative measurement of Carcinoembryonic Antigen (CEA) immune complexes (CEA-IgM).

EXPLANATION

Colon-IC belongs to a novel generation of in vitro diagnostics based on the detection of CEA as circulating Immune Complex (IC). Colon-IC is a highly specific and sensitive ELISA assay for Colorectal cancer (CRC) detection designed to measure CEA-IgM in patient sera. The amount of CEA-IgM is expressed in Arbitrary Units (AU/mL), using a CEA-IgM standard of human origin as a reference. The measurement of CEA-IgM offers an increased sensitivity of early stage (stage 1) CRC, without compromising specificity with respect to the well-established serum CRC biomarker assays such as the quantitative determination of free CEA (1). Furthermore, CEA-IgM is a complementary biomarker to free CEA, thus when used together the overall sensitivity increases to 64% (1).

PRINCIPLE OF THE TEST

Standard Calibrators and specimens are incubated in parallel with anti-CEA antibodies coated to the wells of a microtiter plate. The immune complexed CEA-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

XG006-PL: 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-CEA.

XG006-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 filter
Distilled or deionized water

STORAGE CONDITIONS

Storage at 4°C:

XG006-PL, XG-CH3, XG-ST3, XG-DB5*, XG-WB2*

Storage at -20°C:

XG-EA, XG006-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 **XG006-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 (2). Biosafety Level 2 (3) or other appropriate biosafety practices (4, 5) 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 Colon-IC assay.

Serum specimens should be collected aseptically, avoiding hemolysis if possible.

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



PRODUCT DATA SHEET

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 XG006-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 XG006-Calibrator vial. Also dispense 100 μL /well of XG-DB5 dilution buffer as blank, in duplicate.
6. Dispense 100 μL /well of a 50-fold 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 CEA-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 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



PRODUCT DATA SHEET

with OD values exceeding the upper calibration limit should be further diluted and re-measured.

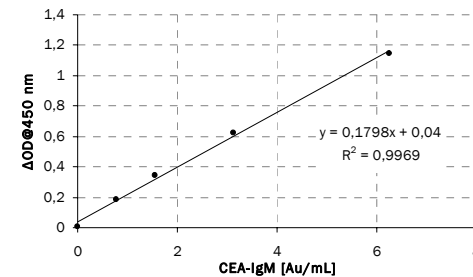


FIG. 1A: range of linearity of a typical standard curve for CEA-IgM after 15 minutes of substrate incubation and addition of stop solution

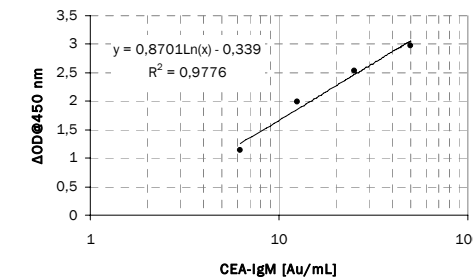


FIG. 1B: range of linearity of a typical semi-logarithmic standard curve for CEA-IgM after 15 minutes of substrate incubation and addition of stop solution

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 CEA-IgM cut-off value was 200 AU/mL for differentiating CRC from healthy subjects (1).

SPECIFIC PERFORMANCE CHARACTERISTICS

The linear range of the assay is 0-50 AU/mL. The sample with values above 50 AU/mL should be further diluted and re-measured.

REFERENCES

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