



## PRODUCT DATA SHEET



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## PRODUCT DATA SHEET

### SCCA-Lisa

**XG004**

*ELISA Kit for the detection and quantification of Squamous Cell Carcinoma Antigen variants (SCCA) in biological samples*

Rev. 02/2009

#### INTENDED USE

SCCA-Lisa is an enzyme linked immunosorbent assay (ELISA) for the quantitative measurement of Squamous Cell Carcinoma Antigen (SCCA) variants in biological samples.

#### SUMMARY AND EXPLANATION OF THE TEST

The SCCA-Lisa is a sandwich ELISA for the determination of SCCA in biological samples, performed on a 96 well multi-strip plate pre-coated with an affinity purified anti-SCCA antibody. SCCA has been identified in the past as a serological marker for squamous cell carcinomas of the uterine cervix, vulva, lung, head and neck, and esophagus; thus providing aid in the management of patients with squamous cell carcinoma (1-3). Recent studies with SCCA-Lisa have also indicated its usefulness in the diagnosis of Hepatocellular carcinoma (HCC), suggesting that the use of SCCA in combination with other known markers may provide an increased accuracy of HCC diagnosis in clinical practice (4-11).

#### PRINCIPLE OF THE TEST

Standard Calibrators and specimens are incubated in parallel with anti-SCCA variants antibodies coated to the wells of a microtiter plate. The samples, and the standard calibrators, are diluted and added to the wells. After incubation, the plate is washed to remove unbound proteins. The SCCA variants are revealed by the use of a biotinylated anti-SCCA antibody followed by incubation with a peroxidase enzyme-conjugated streptavidin. The addition of the enzyme substrate leads to the production of a colored product. The developed color is proportional to the amount of the analyte in the sample. The sample SCCA concentration can be easily calculated from the standard curve by interpolation.

#### REAGENTS AND MATERIALS PROVIDED

**XG004-PL:** 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-SCCA.

**XG004-SC:** 100 µL of 160 ng/mL Recombinant SCCA standard solution in PBS containing 1% BSA. The solution contain Proclin as preservative. (Red cap)

**XG004-BA:** 250 µL of 40X concentrated biotin-conjugated rabbit anti-SCCA in PBS containing 1% BSA (Green Cap)

**XG-ES:** 1.3 mL of 10X concentrated Enzyme-conjugated streptavidin in stabilizer solution. (Yellow cap). The solution contains Proclin as preservative.

**XG-CH3:** 10 mL of TMB (3,3',5,5'-Tetramethylbenzidine) chromogen solution ready to use.

**XG-ST3:** 10 mL of 1N HCl Stop solution ready to use.

**XG-DB5:** 10 mL of 5X concentrated Dilution Buffer solution. Once diluted, the working solution contains 1% BSA and 0.05% Tween 20 in PBS. The solution contain Proclin as preservative.

**XG-WB2:** Two tablets of lyophilized Washing Buffer. White powder. 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 or 650 ± 20 nm filter

Distilled or deionized water

#### STORAGE CONDITIONS

**Storage at 4°C:**

XG004-PL, XG-CH3, XG-ST3, XG-ES, XG-DB5\*, XG-WB2\*

**Storage at -20°C:**

XG004-SC, XG004-BA.

Avoid repeated freeze and thaw cycles

(\* ) Must be used within one month of reconstitution

#### EXPIRATION DATE

Expiration date printed on the kit indicates limits of stability.

#### WARNINGS - POTENTIAL BIOHAZARDOUS MATERIALS:

No dangerous or toxic components are present.

For research use only. Not intended for diagnostic or therapeutic purposes in humans and animals.

## 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

- 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 500mL of deionized water.
- Prepare the required amount of XG004-SC standard solution diluting 20-fold in XG-DB5 dilution buffer to a final concentration of 8 ng/mL.
- Prepare the required amount of XG004-BA biotin-conjugated secondary antibody solution diluting 40-fold in XG-DB5 dilution buffer.
- Prepare the required amount of XG-ES diluting 10-fold in XG-DB5 dilution buffer.

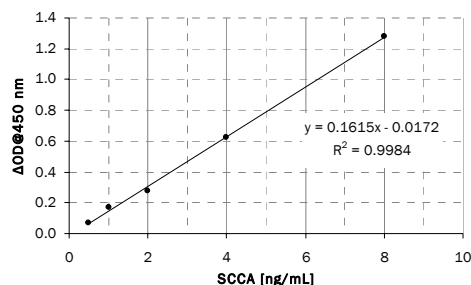
#### ASSAY PROTOCOL

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 three times with XG-WB2 washing buffer (300 µL/well).
5. Dispense 100 µL/well of XG004-SC standard solution (in duplicate) starting from 8 ng/mL and performing in-plate 2-fold serial dilutions to a final concentration of 0.5 ng/mL, in order to obtain a 5-point calibration curve. Dispense 100 µL/well of opportunely diluted sample in duplicate. Use XG-DB5 dilution buffer as diluent. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank, in duplicate.
6. Incubate 1 hour at room temperature.
7. Wash three times with XG-WB2 washing buffer (300 µL/well).
8. Add 100 µL/well of diluted XG004-BA biotin-conjugated secondary antibody.
9. Incubate 1 hour at room temperature.
10. Wash three times with XG-WB2 washing buffer (300 µL/well).

11. Add 100 µL/well of diluted XG-ES enzyme-conjugated streptavidin solution.
12. Incubate 1 hour at room temperature.
13. Wash three times with XG-WB2 washing buffer (300 µL/well).
14. Apply 100 µL/well of XG-CH3 chromogen solution.
15. Allow color to develop for 10-15 min at room temperature in the dark and measure OD values of each well using an ELISA plate reader with a 650nm filter or, alternatively, apply 100µL/well of XG-ST3 Stop Solution and measure OD values of each well using an ELISA plate reader with a 450nm filter. Stopped reaction should be read within 1 hour.
16. 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 SCCA concentration in the biological sample can be calculated directly from the standard curve by interpolation. The value obtained must be multiplied by the dilution factor.



**FIG. 1:** Range of linearity of a typical standard curve for SCCA. After 15 minutes, the reaction was stopped by adding 100µL/well of stop solution. Absorbance was read at a wavelength of 450 nm.

#### QUALITY CONTROL

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

#### SPECIFIC PERFORMANCE CHARACTERISTICS

##### Specificity

This assay recognizes human SCCA protein isoforms (SCCA-1, SCCA-2, SCCA-PD).

##### Linearity

The range of detectability for this assay corresponds to the range of linearity of a typical standard curve and is included between 0.5 and 8 ng/mL of SCCA proteins.

The SCCA concentrations lower than 0.5 ng/mL are undetectable, while SCCA levels higher than 8 ng/mL need further dilutions to be included in the range of linearity of the curve and to be correctly detected.

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