1.0 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.

2.0 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).

3.0 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.

4.0 REAGENTS AND MATERIALS PROVIDED

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

**XG-SC1**: Lyophilized Recombinant SCCA standard solution. Exact concentration on the label.

**XG-BA1**: 14 mL of biotin-conjugated rabbit anti-SCCA in stabilizer solution. Ready to use.

**XG-ES1**: 14 mL of Enzyme-conjugated streptavidin in stabilizer solution. Ready to use.

**XG-CH7**: 14 mL of TMB (3,3′,5,5′-Tetramethyl-benzidine) chromogen solution. Ready to use.

**XG-ST7**: 14 mL of H₂SO₄ 0.3M Stop solution. Ready to use.

**XG-DB5**: 14 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-WB3**: 100 mL of Concentrated Washing Buffer solution 10X. Dilute in deionized water.

5.0 MATERIAL AND EQUIPMENT REQUIRED

Precision pipettes with disposable tips.
Microplate washer.
Microplate readers with a 450 ± 20 nm filter.
Distilled or deionized water.

6.0 STORAGE CONDITIONS

Store at 2-8°C.
Expiration date is printed on each component and on the packaging label.

7.0 WARNINGS AND PRECAUTIONS

7.1 SAFETY REGULATIONS

- Do not pipette by mouth. Use disposable gloves and eyes protection while managing samples and during the test. Wash hands carefully once the test is completed.
- The following reagents contain low concentration of dangerous and stinging substances:
  - Washing buffer (XG-WB3) contains detergent;
  - Dilution buffer (XG-DB5) contains Proclin;
  - Chromogen solution (XG-CH7) is acid.
- If a reagent comes into contact with skin or eyes rinse abundantly with water;
- Not disposable equipment must be sterilized after use, in an autoclave at 121°C for 1 hour; disposable equipment must be autoclaved or incinerated;
- Sulphuric acid contained in the stop solution (XG-ST7) used to wash glassware are corrosive; those substances must be used with caution. In case of contact with skin and eyes rinse abundantly with water;
- Neutralized acid and other liquid/fluid waste must be disinfected adding sodium hypochlorite in a volume sufficient to obtain at least 1% final concentration. An exposure to sodium hypochlorite 1% for at least 30 minutes should be sufficient to ensure effective disinfection. The spill of potential infected materials must be cleaned immediately with blotting paper and the contaminated zone should be disinfected with sodium hypochlorite 1%
before continuing to work. If case of spills containing acid, sodium hypochlorite should not be used before the area has dried. All materials used to clean accidental spills, including gloves, must be discarded as potentially infected waste. Do not put materials containing sodium hypochlorite in autoclave.

7.2 TECHNICAL PRECAUTIONS

- Store at 2-8°C.
- Bring reagents and samples to room temperature (18-30°C) before use. Put reagents at recommended storage temperature immediately after use.
- Open the strip bag after at least 30 minutes at room temperature.
- Do not use reagents after expiration date. Avoid microbial contamination of reagents, since it can reduce product validity and generate incorrect results.
- Do not change the procedure or replace reagents with those of other manufacturers or other lots. Do not reduce recommended incubation time.
- All glassware used during the test must be cleaned with hydrochloric acid 2M and rinsed accurately with distilled or deionized water.
- Avoid the use of auto-defrost freezers for samples storage.
- Do not expose reagents to strong light or hypochlorite gas during storage and incubation periods.
- Avoid drying of the wells during the test.
- It is important to use specific pipettes in order to avoid cross-contamination of reagents.
- Avoid spilling the edge of the wells with the enzyme-conjugated secondary antibody. Do not blow on the microplate.
- Sometimes enzyme immunoassays show an “edge effect” on the wells' edges; this can be minimized by increasing the moisture during the incubation phases. Plates must be covered and incubated in the dark. Alternatively, they can be incubated in a suitable analyzer. For more details consult the dedicated instrument operative manual. Do not use CO₂ incubators.
- Before reading the plate, make sure that the bottom is clean and dry and there are no air bubbles on the surface of the liquid.
- Strongly hemolyzed specimens, incompletely coagulated serum samples or microbial contaminated samples can generate errors.
- Read the operative manual of each instrument used, with particular attention to the following aspects:
  - Installation and particular requirements;
  - Principle of operation, instructions, precautions, risks;
  - Manufacturer specifics and instrument performance;
  - Maintenance and technical support.

8.0 SPECIMEN COLLECTION AND PREPARATION

The use of serum samples are recommended in the SCCA-LISA assay. Serum specimens should be collected aseptically, avoiding hemolysis when possible. Specimens should be stored at 2-8°C if the assay is performed within 24 hours from collection. In all other cases, samples should be frozen. When frozen, specimens should be mixed thoroughly after thawing to ensure consistency in the results. Avoid repeated freezing and thawing. Samples showing particulate matter, erythrocytes, or turbidity must be clarified by centrifugation before testing.


9.0 INSTRUCTIONS FOR USE

9.1 TECHNICAL 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 touching the edge of the wells both before and after filling.
• Standards and samples should be tested in duplicate.
• Run a separate standard curve for each assay.
• For each assay, use only coated wells from the same reagent batch. Do not mix reagents from different lots.
• Perform the incubations in a covered box containing a wet paper towel in order to prevent evaporation.

9.2 REAGENTS PREPARATION

• Reconstitute XG-SC1 with 1500 μL of deionized water. Reconstituted calibrators must be stored at 2-8°C and used by one weeks.
• Prepare the required amount of XG-DB5 dilution buffer by diluting the concentrated solution 5-fold in deionized water. Storage at 2-8°C may generate crystals in concentrated solution; in this case heat the bottle at 37°C with mixing to dissolve. Reconstituted dilution buffer must be stored at 2-8°C and used by one month.
• Prepare the required amount of XG-WB3 washing buffer by diluting the concentrated solution 10-fold in deionized water. Reconstituted washing buffer must be stored at 2-8°C and used by one month.

9.3 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-WB3 washing buffer (300 μL/well).
5. Dispense 100 μL/well of XG-SC1 standard solution (in duplicate) performing in-plate 2-fold serial dilutions 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-WB3 washing buffer (300 μL/well).
8. Add 100 μL/well of XG-BA1 biotin-conjugated secondary antibody.
9. Incubate 1 hour at room temperature.
10. Wash three times with XG-WB3 washing buffer (300 μL/well).
11. Add 100 μL/well of XG-ES1 enzyme-conjugated streptavidin solution.
12. Incubate 1 hour at room temperature.
13. Wash three times with XG-WB3 washing buffer (300 μL/well).
14. Apply 100 μL/well of XG-CH7 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-ST7 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.

10.0 RESULTS

10.1 PROCESSING OF THE RESULTS

a. Calculate the average of duplicate readings for each standard calibrator and sample.
b. Subtract the absorbance of the blank from the readings of each standard and sample.
c. Create a standard curve by plotting the known concentrations of standard (X) vs. the absorbance of standard (Y).
d. Calculate the sample SCCA concentration by interpolation of the linear curve formula.
e. Multiply by the dilution factor to obtain the concentration of the undiluted samples.

![](image)

**Figure 1**: Typical standard curve for SCCA. After 10 minutes the reaction was stopped by adding 100µL/well of stop solution. Absorbance was read at a wavelength of 450 nm.

10.2 QUALITY CONTROL

The intra- and inter-assay coefficient of variation was determined on four typical standard curves and the result was <15%. For optimal performance, the absorbance of the blank should be <0.25 OD @450nm.

It is recommended that each laboratory tests appropriate quality control samples in each run to ensure the accuracy of all reagents and procedures.

11.0 ASSAY CHARACTERISTICS

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

11.1 RANGE

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.
REFERENCES


