

SCCA-Lisa

XG004

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

The Squamous Cell Carcinoma Antigen (SCCA), a member of the ovalbumin family of serine proteinase inhibitors, is a tumor-associated antigen and a serological and histological marker for many squamous cell carcinomas (SCC). Increased SCCA protein level in the blood has been demonstrated to have clinical relevance as circulating biomarker for advanced squamous cell carcinomas of the uterine cervix, lung, esophagus, head and neck and inverted papilloma (Ref. 1, 2). In addition, SCCA proteins (SCCA1, SCCA2, and SCCA-PD, a novel variant) have been reported to be over-expressed in hepatocellular carcinoma (HCC) in at least 85% of HCC livers as detected by immunohistochemistry (Ref. 3, 4) (FIG.1). Clinical studies on cervical squamous cell carcinoma, for example, show that the percentage of patients with elevated circulating levels of SCCA increases throughout tumor progression, in particular from approximately 12% at stage 0 to more than 90% at stage IV (Ref. 5). SCCA circulating levels fall after tumor resection, but rise again in approximately 90% of patients with recurrent disease.

Furthermore, measurement of post-treatment SCCA levels is useful in monitoring the response to therapy and in predicting tumor recurrence and metastasis (Ref. 6).

SCCA-Lisa is a sensible, specific and well standardized Elisa Kit for in vitro detection and quantification of SCCA proteins (SCCA 1, SCCA 2, and SCCA -PD) from tissue/ cellular culture media, tissue/ cellular protein extracts, biological samples, such as plasma or serum (Ref. 7).

Principle

SCCA is measured by using microtitre plate coated with a capturing antibody. After washing the plate to remove unbound proteins, SCCA is detected by incubating with a biotinylated revealing antibody. After a final step with HRP-conjugated streptavidin, the plate is developed with a solution of peroxidase substrate which produces a coloured end product.

As the concentrations of capturing and detecting antibody are fixed, the colour generated is proportional to the concentration of analyte in the sample.

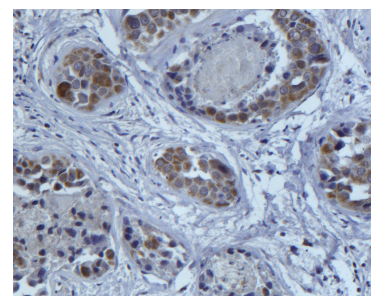
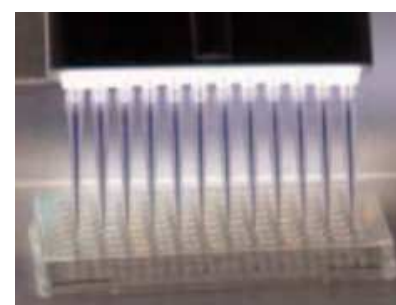


FIG.1: Histochemical appearance of Hepatocellular Carcinoma (HCC) nodules stained with anti-SCCA variants antibody (HepAb, XEPTAGEN)

APPLICATION: SCCA determination in culture medium from SCCA-transfected HEPG2 cells

Culture medium samples were diluted 10, 50 and 100 times. The SCCA standard solution was also serially diluted in order to have a reference curve in which SCCA concentration ranges from 10 to 0,156 ng/mL. All dilutions were performed in dilution buffer and dispensed in duplicates. The assay was carried out as described and the results summarized in the figure below:

Sample	Conc./Dil.	A ₄₀₅	ΔA ₄₀₅	ng/mL
Blank		0,396		
SCCA (ng/mL)	10	0,856	0,460	
"	5	0,789	0,393	
"	2,5	0,742	0,346	
"	1,25	0,683	0,287	
"	0,625	0,604	0,208	
"	0,313	0,526	0,130	
"	0,156	0,474	0,078	
SN HepG2 (dil.)	10	0,637	0,241	8,5
"	50	0,459	0,063	7,2
"	100	0,409	0,013	8,7



SCCA-Lisa Kit multi-strip Assay-Plate

The assay standard linearity range extends, as expected, from 0.156 to 2.5 ng/mL of SCCA. SCCA concentrations in culture medium, serum, plasma or other biological fluids are calculated by interpolation.

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References

1. Uemura Y et al, Int J Cancer, 89:368-377 (2000)
2. Cataltepe S et al, Clin Chem Acta, 295:107-127 (2000)
3. Ruvoletto MG et al, J Hepatology, 36: 83 (2002)
4. Pontisso P et al, Brit J Cancer, 90(4):833-837 (2004)
5. Duk JM et al, Gynecol Oncol, 39:186-194 (1990)
6. Lara PC et al, Cancer, 76:758-764 (1995)
7. Quarta S et al, Digestive and Liver Disease, 36:A19 (2004)

Specifications

Materials provided:

- **XG004-PL:** 96 wells multi-strip Assay-Plate, pre-coated with affinity purified rabbit anti-SCCA IgG.
- **XG004-SC:** recombinant SCCA standard solution, 100 ng/mL in PBS containing 1% BSA, 200 μ L.
- **XG004-BA:** Affinity purified biotin-conjugated rabbit anti-SCCA IgG, 200 μ g/mL in PBS, 300 μ L.
- **XG-ES:** Enzyme-conjugated streptavidin solution.
- **XG-CH2 Chromogen solution.**
- **XG-SB Enzyme substrate solution.**
- **XG-DB Dilution Buffer.**
- **XG-WB Washing Buffer.**

Equipment required:

Microplate washer and microplate reader

Brief description of procedure

Calibration curve and samples: Dispense 100 μ L/well of **XG004-SC** standard (typical titration curve: 10 ng/mL, 5ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.31 ng/mL, 0.16 ng/mL) and diluted samples. Incubate 1h at room temperature. Wash 6x with **XG-WB** washing buffer.

Secondary Antibody: Dilute the **XG004-BA** in **XG-DB** to get a final concentration of 5 μ g/mL and dispense 100 μ L/well.

Incubate 1h at room temperature. Wash plate 6x with **XG-WB** washing buffer.

Streptavidin-HRP solution: Dispense 100 μ L/well of previously diluted **XG-ES** revealing molecule solution. Incubate 1h at room temperature. Wash plate 6x with **XG-WB** washing buffer.

ABTS substrate solution: Apply 200 μ L/well of freshly prepared ABTS substrate (**XG-CH2**). Allow colour to develop in the dark at R.T. for about 10-15 minutes and measure OD values of each well using an ELISA plate reader set to 405 nm. Plot blank-subtracted OD405 versus standard concentration. Read unknowns from the standard curve. A typical standard curve is shown in FIG.2.

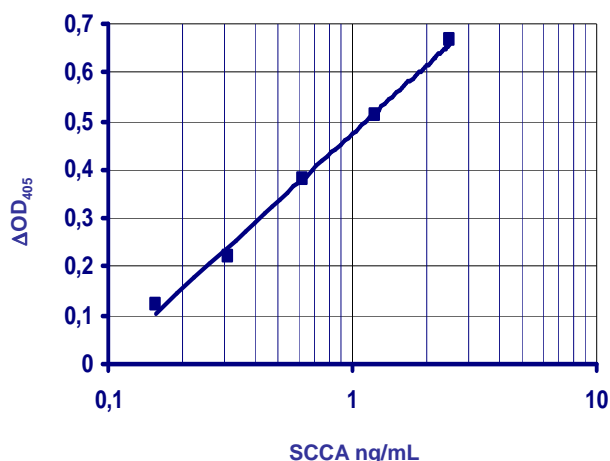


FIG.2: Range of linearity of a typical standard curve for SCCA after 15 min substrate incubation.

Code XG004 96 well assay

For in vitro use only

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