Version 16-04/24

User's Manual

Human SCCA2(Squamous Cell Carcinoma Antigen 2) ELISA Kit

REF **DEIANS074**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For quantitative detection of human SCCA2 in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP -Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of target can be calculated.

Reagents And Materials Provided

1. Pre-coated Microplate,8×12 wells, 4°C/-20°C(6 months)
2. Standard (lyophilized), 2 vials, 4°C/-20°C(6 months)
3. Standard/Sample Diluent Buffer, 20mL, 4°
4. Biotinylated Antibody (100×), 120µL, 4°C/-20°C(6 months)
5. Biotinylated Antibody Diluent, 12mL, 4°C
6. Streptavidin-HRP (100×), 120µL, 4°C/-20°C(6 months)
7. HRP Diluent, 12mL, 4°C
8. Wash Buffer (25×), 20mL, 4°C
9. TMB Substrate Solution, 10mL, 4°C(Keep away from light)
10. Stop reagent, 6mL, 4°C
11. Plate Covers, 2
12. Instruction manual

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. Incubator
3. Centrifuge
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

2- 8°C (use within one week)

-20°C (for long-term storage, the reagents should be kept at the temperature indicated on the label)

Avoid repeated freezing and thawing, do not use after expiration date.

Specimen Collection And Preparation

1. Serum: Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin. Store at -20°C or -80°C, and avoid repeated freezing and thawing.

2. Plasma: Collect plasma using EDTA-Na_2 as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples. Store at -20°C or -80°C, and avoid repeated freezing and thawing.

3. Tissue Homogenates: As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.0-7.2). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5-10 minutes at 5000×g to get the supernatant.

4. Cell culture supernatant: Centrifuge the supernatant at 1000 ×g for 20 min at 2-8°C to remove insoluble impurities and cell debris. Collect the clarified supernatant and perform the assay immediately. Store at -20°C or -80°C, and avoid repeated freezing and thawing.

5. Urine: Please collect the first morning urine (midstream), or 24-hour urine. Collect urine sample in sterile tube and then centrifuge at 2000×g for 15 minutes. Collect supernatant as last.

6. Saliva: Collect saliva sample in sterile tube and then centrifuge at 1000×g at 2-8°C for 15 minutes. Collect supernatant as last. Or store at -20°C.

7. Other Biological Fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples used within 1 week can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test. If the concentration of the detected substance in your sample is higher than the highest value of the standard, please make an appropriate multiple dilution according to the actual situation (it is recommended to do a preliminary experiment to determine the dilution multiple).

Reagent Preparation

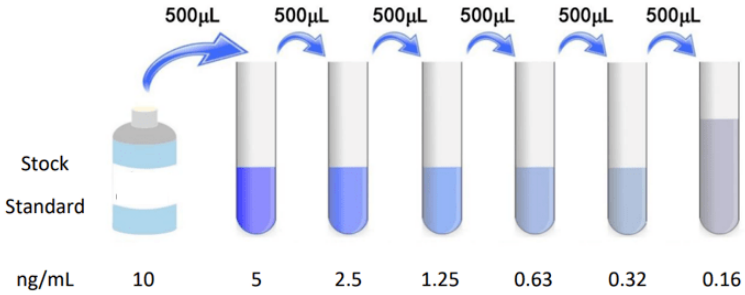
Bring all reagents and samples to room temperature for 30 minutes before use.

1. Wash Buffer:

Dilute 25× Wash Buffer to 1× Wash Buffer with deionized or distilled water (The recommended resistivity of deionized or distilled water is 18MΩ). Put unused solution back at 2-8°C.

2. Standards:

a. Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly. Thereafter, doubling dilutions were performed to 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.63ng/mL, 0.32ng/mL, 0.16ng/mL. The standard dilution (0pg/mL) is a blank hole. **Note: It is best to use Standard Solutions within 15 min.**



3. Preparation of Biotinylated Antibody Working Solution:

a. Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

b. Dilute the Biotinylated Antibody with Biotinylated Antibody Diluent at 1:100 and mix them thoroughly. (i.e. Add 1μl Biotinylated Antibody into 99μl Biotinylated Antibody Diluent.)

4. Preparation of Streptavidin-HRP Working Solution:

Prepare it within 30 minutes before experiment.

a. Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

b. Dilute the Streptavidin-HRP with HRP Diluent at 1:100 and mix them thoroughly. (i.e. Add 1μl of Streptavidin-HRP into 99μl of HRP Diluent.)

5. TMB Substrate Solution

Use a pipette to aspirate the required volume of solution, do not pour the remaining solution back into the reagent bottle.

Assay Procedure

Note:

1. Bring all materials and reagents to room temperature before use. Before use, mix all reagents thoroughly, taking care not to generate any foam.
2. The user should calculate the number of samples that may be used throughout the test. Please reserve enough samples in advance.
3. Please estimate the concentration before measurement. If these values are not within the range of the standard curve, the user must determine the optimal sample dilution for their particular experiment.

Procedure:

1. Add Samples: Add 100µl of standards and properly diluted sample into test sample wells. Be careful not to get air bubbles. Add the solution at the bottom of each well without touching the sidewall, shake gently to mix. Seal the plate with a cover and incubate at 37°C for 80 minutes.

2. Wash: Remove the cover, and wash plate 3 times with 200 µl Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time. Shake off the liquid in the microplate (or wash the plate with a plate washer). After the last wash, pat the plate dry on absorbent paper.

3. Biotinylated Antibody Working Solution: Add 100µl Biotinylated Antibody Working Solution into wells. Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 50 minutes.

4. Wash: Remove the cover, and wash plate 3 times with 200 µl Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time. Shake off the liquid in the microplate (or wash the plate with a plate washer). After the last wash, pat the plate dry on absorbent paper.

5. Streptavidin-HRP Working Solution: Add 100µl Streptavidin-HRP Working Solution into wells. Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 50 minutes.

6. Wash: Remove the cover, and wash plate 3 times with 200 µl Wash Buffer, and let the wash buffer stay in the wells for 1-2 minute each time. Shake off the liquid in the microplate (or wash the plate with a plate washer). After the last wash, pat the plate dry on absorbent paper.

7. TMB Substrate: Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 20 minutes. (Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.) (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when the standard well shows clear gradient blue.)

8. Stop: Add 50µl Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

9. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Calculation

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation.

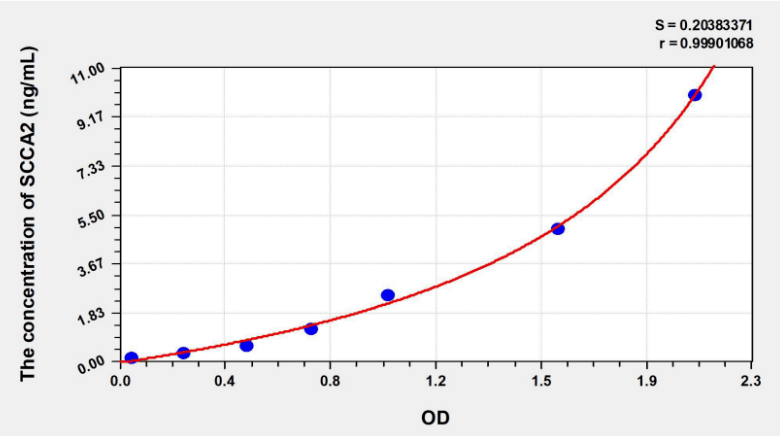
The OD value of the used sample can be used to calculate the sample concentration on the standard curve. Multiply by the dilution factor, which is the actual concentration of the sample.

Typical Standard Curve

This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard

curve as per experiment by themselves.

Concentration (ng/mL)	OD	Corrected OD
10	2.19	2.107
5	1.688	1.605
2.5	1.067	0.984
1.25	0.782	0.699
0.63	0.548	0.465
0.32	0.319	0.236
0.16	0.124	0.041
0	0.083	0.000



Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Specificity

This assay has high sensitivity and specificity for the detection of recombinant or natural SCCA2.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of SCCA2 and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8	1:16
Serum(n=5)	79-92%	80-93%	81-98%	89-102%
EDTA Plasma(n=5)	89-97%	86-95%	81-93%	91-103%
Heparin Plasma(n=5)	85-99%	82-96%	92-101%	82-90%

Recovery

Matrices listed below were spiked with certain level of SCCA2 and the recovery rates were calculated by comparing the measured value to the expected amount of SCCA2 in samples.

Sample	Recovery Range (%)	Average (%)
Serum(n=5)	82-95%	88%
EDTA Plasma(n=5)	84-97%	90%
Heparin Plasma(n=5)	79-93%	86%

Precautions

1. There may be some substances in the newly opened well of the enzyme label plate, which is a normal phenomenon and will not have any influence on the experimental results
2. The Wash Buffer will be salted out when kept at low temperature, and can be warmed in the water bath (< 40°C) to help dissolve when diluted.
3. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
4. Do not use kit components beyond their expiration date.
5. Use fresh disposable pipette tips for each transfer to avoid contamination.
6. Storage TMB reagents avoid light.
7. Biotinylated Antibody and Streptavidin-HRP are small and may disperse throughout the tube during shipping. Centrifuge at 1000×g for 1 minute before use to allow liquid from the tube wall or cap to settle to the bottom of the tube. Carefully pipette 4-5 times to mix the solution before use.
8. This kit is for research use only
9. Reagents should be treated as hazardous substances, handled with care and disposed of properly.
10. Wear gloves, lab coats, and protective glasses at all times to avoid skin and eye contact with the termination fluid and TMB. In case of contact with TMB, wash it thoroughly with water.