

## Instructions for Use



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# SCCA2 ELISA

Enzyme linked immunosorbent assay (ELISA) is intended for the quantitative detection SCCA2 in serum.

**REF** 30256207

 96

Distributed by: **IBL International GmbH**  
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22335 Hamburg, Germany

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## SCCA2 kit

# IMMQUA SCCA2

**[Intended use]**

IMMQUA SCCA2 (squamous cell carcinoma antigen 2) is an enzyme linked immunosorbent assay (ELISA) intended for the quantitative detection SCCA2 in serum.

**[General precautions]**

1. Product is a research use only (RUO) reagent that should not be used for diagnostic purposes.
2. The use of this product for purposes other than those described in the package insert is not guaranteed.
3. Read the instructions for use of the kit carefully before use.
4. Product contains sodium azide as a preservative. If the substance gets into the eyes, mouth, or comes in contact with skin, take first aid measures such as washing it off thoroughly with water, and consult a doctor if necessary.

**[Kit Components]****(1) Antibody-coated Plate**

Rat Anti-SCCA Monoclonal Antibody [SCCA Antibody]

**(2) Standard****(3) Sample Diluent****(4) POD-conjugate**

Peroxidase-labeled rat anti SCCA2 monoclonal antibody [POD-labeled antibody]

**(5) Conjugate Solvent****(6) Color Reagent A (TMBZ)**

3,3',5,5'-Tetramethylbenzidine dihydrochlorate dehydrate

**(7) Color Reagent B (Hydrogen peroxide)****(8) Stop Solution****(9) Wash Concentrate****(10) Control Material**

Accessory: Plate seal

**[Assay principle]**

This product is a reagent for measuring SCCA2 in serum by ELISA. Samples containing SCCA2 react in a 96-well microplate (solid phase) coated with anti-SCCA2 antibody. After washing, POD-labeled anti-SCCA2 antibody is added and allowed to react, forming an immune complex via SCCA2 in the sample. Unreacted POD-labeled anti-SCCA2 antibody is removed by washing, and then a chromogenic substrate is added to initiate the enzyme reaction. The concentration of SCCA2 in the sample, which is bound to the solid-phase antibody, is proportional to the color intensity generated by the reaction between POD and the chromogenic substrate. By measuring this color intensity, the concentration of SCCA2 can be determined.

**[Operational precautions]****Properties of the test sample and collection method**

1. Use fresh serum as much as possible for the sample. If sample is to be stored for a long period of time, keep it frozen at -20°C or less, and avoid repeated freezing and thawing.
2. SCCA2 is also found in the skin epidermis, saliva, sweat, and other body fluids. If samples, reagents, instruments, etc.

that have been contaminated or contacted with these substances are used, the measurement value may increase. Repeat the measurement if unexpectedly high values are observed.

3. When handling specimens, reagents, instruments, etc., always wear gloves and a mask.

**Cross-reactivity**

The kit does not react with SCCA1.

**Interfering substances and drugs**

1. Hemoglobin does not affect the test results up to 500 mg/dL.
2. Bilirubin does not affect the test results up to 50 mg/dL.
3. Rheumatoid factor does not affect the test results up to 500 IU/mL.
4. Chyle does not affect the test results up to 3000 formazin turbidity units.

**[Operation method]****Preparation of reagents**

1. Antibody-coated plate: (1) Use the antibody-coated plate as provided.
2. Standard solution: (2) Prepare the standard by diluting it to 0.2, 0.1, 0.05, 0.025, 0.0125, 0.0063 ng/mL with (3) Sample Diluent, and use accordingly.
3. Sample Diluent: (3) Use the sample diluent as provided.
4. Labeled antibody solution: (4) Dissolve the POD-conjugate with one vial of (5) conjugate solvent. After dissolution, tightly seal and store at 2–8°C. It can be used for up to 2 weeks.
5. Chromogenic substrate solution: (6) Mix equal volumes of color reagent A and (7) color reagent B to prepare the chromogenic substrate solution. After mixing, store at 20–30°C and use within 60 minutes.
6. Stop solution: (8) Use the stop solution as provided.
7. Wash solution: Add 4 volumes of purified water to 1 volume of (9) wash concentrate, mix well, and use as the wash solution.
8. Control material: (10) Use the control material as supplied.

**Instruments and equipment required**

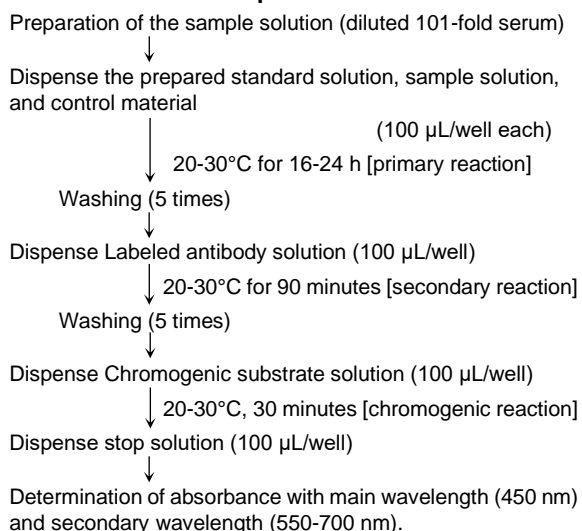
1. Micropipette or measuring pipette (for preparation and dispensing of reagents and samples)
2. Test tubes (for sample dilution)
3. Vortex mixer
4. 1 L graduated cylinder (for preparation of wash solution)
5. Repetitive/dispenser pipette (for dispensing reagents)
6. Microplate washer (for washing)
7. Incubator (20–30°C; for incubation)
8. Microplate reader

**Measurement (Operation) method**

Take each reagent out of the refrigerator at least 30 minutes before use and allow it to reach 20–30°C prior to use. An example of the procedure is provided below.

1. Add 10  $\mu\text{L}$  of serum to 1 mL of sample diluent to prepare the test sample solution (101-fold dilution).
2. Dispense exactly 100  $\mu\text{L}$  each of the sample diluent (0 ng/mL SCCA2 concentration), prepared standards, sample solutions, and control material into the designated wells of the antibody-coated plate. Perform all measurements in duplicate.
3. Incubate at 20–30°C for 16–24 hours. During incubation, cover the wells with a plate seal to prevent contamination from dust, debris, skin flakes, saliva, sweat, or other bodily fluids.
4. Remove the reaction mixture from the wells using an aspirator, etc.
5. Add 400  $\mu\text{L}$  of wash solution to each well of the antibody-coated plate, then remove the wash solution using an aspirator, etc. Repeat this washing step five times.
6. After washing, thoroughly remove any residual wash solution from the wells (e.g., by gently tapping the plate onto a paper towel) before proceeding to the next step.
7. Add 100  $\mu\text{L}$  of labeled antibody solution to each well of the antibody-coated plate.
8. Cover the wells with a plate seal and incubate at 20–30°C for 90 minutes.
9. Perform the washing steps as described in steps 4–6.
10. Add 100  $\mu\text{L}$  of chromogenic substrate solution to each well of the antibody-coated plate.
11. Incubate at 20–30°C for 30 minutes. During incubation, cover the plate with a plate cover or wrap to prevent contamination from dust or debris.
12. Add 100  $\mu\text{L}$  of stop solution to each well of the antibody-coated plate.
13. Measure the absorbance of all wells at 450 nm (primary wavelength) and 550–700 nm (reference wavelength). Perform the measurement within 120 minutes after stopping the reaction, under conditions of 20–30°C.

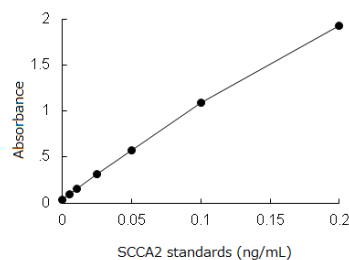
#### Outline of measurement procedure



#### Calculation of measured results

1. Subtract the absorbance at the reference wavelength (550–700 nm) from the absorbance at the primary wavelength (450 nm).
2. Plot the concentration and absorbance of each standard solution to draw calibration curve.
3. Determine the concentration of SCCA2 in the sample solution (ng/mL) from the calibration curve.
4. Calculate the SCCA2 concentration in serum by multiplying the determined concentration of the sample solution by the dilution factor of the specimen.
5. For each sample, use the average of the concentrations obtained from duplicate measurements as the test result.
6. If a specimen exceeds the measurement range, dilute the sample appropriately with sample diluent and remeasure.

Example of calibration curve



#### [Summary and Explanation]

SCCA (squamous cell carcinoma antigen) is a serine protease inhibitor belonging to the serpin superfamily and is a protein with a molecular weight of approximately 45 kDa, mainly produced by epithelial cells. SCCA has two isoforms, SCCA1 (SERPIN B3) and SCCA2 (SERPIN B4), which, despite exhibiting a very high amino acid sequence homology of 91%, are independent gene products transcribed from different genetic loci. The central mechanism of allergic inflammation is the Th2-type immune response, in which interleukin-13 (IL-13), one of the Th2 cytokines, is known to play an important role. SCCA2 was identified as one of the molecules whose expression is upregulated in airway epithelial cells when stimulated with IL-4 and IL-13, cytokines that play central roles in allergic reactions<sup>1)</sup>.

#### Performance

1. Sensitivity, Accuracy, Simultaneous Reproducibility
  - (1) Sensitivity  
The absorbance of the standard solution increases as the SCCA2 concentration rises, with a difference of 1.20 or more between the 0 ng/mL and 0.2 ng/mL standard solutions.
  - (2) Accuracy  
When a control material with a known concentration is measured, accuracy is within  $\pm 20\%$  if the known SCCA2 concentration is 1.0 ng/mL or higher, and within  $\pm 30\%$  if the concentration is less than 1.0 ng/mL.
  - (3) Reproducibility  
When the same sample is measured three times simultaneously, the C.V. value is less than 15%.
2. Assay range  
0.3-20 ng/mL

#### Reference material for calibration

Human recombinant SCCA2 (internal standard)

#### [Precautions]

##### Handling (hazard prevention)

1. Handle the specimens as potentially infected with HIV, HBV, HCV and others. When assaying, use disposable gloves to reduce the risk of infection and do not aspirate pipetting by mouth.
2. Stop solution is acidic (pH<2), and the specimen diluent contains a trace amount of sodium azide. Avoid direct contact of these solutions on the skin and eyes.
3. If reagents accidentally contact eyes or mouth, wash thoroughly with water as a first-aid measure and see a doctor for further treatment if necessary.
4. Read the latest SDS before use. Please contact us via contact form or e-mail if you wish to receive an SDS.

##### Precaution for Use

1. Do not freeze this product, and store it according to the specified storage conditions. If the reagent has been

accidentally frozen, do not use it, as changes in quality may lead to inaccurate results.

2. Do not use reagents that have passed their expiration date.
3. Do not mix reagents from different lot numbers. Even if the lot numbers are the same, do not combine partially used reagents from different kits.
4. Crystals may be observed at the mouth of the concentrated wash solution bottle; however, performance is not affected and the solution can be used as is.
5. Do not reuse containers or repurpose them for other uses.

### **Precaution for Disposal**

1. Specimens may contain infectious agents such as HIV, HBV, or HCV. Disinfect waste liquids and used instruments by immersing in sodium hypochlorite (effective chlorine concentration 1000 ppm, for at least 1 hour) or glutaraldehyde (2%, for at least 1 hour), or sterilize by autoclaving (121°C for at least 20 minutes).
2. In the event that specimens or specimen-containing solutions are spilled, wipe up thoroughly with 80% alcohol spray or equivalent.
3. The sample diluent contains a small amount of sodium azide. Sodium azide can form explosive metal azides upon contact with lead or copper. For safety, dilute thoroughly with large amounts of water before disposal.
5. The pH of the stop solution is less than 5.8. When disposing, comply with relevant local regulations.
6. When disposing of reagents or instruments, follow relevant laws and local regulations concerning waste disposal and sanitation.

### **[Storage method and shelf life]**

12 months at 2–8 °C.

### **[Packaging unit]**

Product code: 326076529

Product name: IMMQUA SCCA2

Package: 48 assays


















(1) Antibody-coated Plate	1 pcs
(2) Standard	1 mL x 1
(3) Sample Diluent	100 mL x 1
(4) POD-conjugate	12.0 mL x 1
(5) Conjugate Solvent	12 mL x 1
(6) Color Reagent A	6 mL x 1
(7) Color Reagent B	6 mL x 1
(8) Stop Solution	12 mL x 1
(9) Wash Concentrate	100 mL x 2
(10) Control Material	1 mL x 1

Accessories: Plate seal 2 pcs

### **[Reference]**

1. Yuyama N., et al. : Cytokine ; 19: 287-296 (2002)

# Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.-Cat.: / N.º Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλοποιημένο
	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
	Unique Device Identification / Eindeutige Geräteerkennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazena a: / Conservare a: / Armazena em: / Αποθήκευση στους:
	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazena a: 2 - 8°C / Conservare a: 2-8°C / Armazena em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symboles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

**COMPLAINTS:** Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

**WARRANTY:** The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

**LIMITATION OF LIABILITY:** IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.

For further country-specific classifications, please refer to the corresponding safety data sheet.



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