Read this package insert before use.

In-vitro diagnostic reagent

February 2020. (1st ed)

Marketing authorization number 30100EZX00038000

SCCA2 kit

IMMQUA SCCA2

[General precautions]

1. Product is an in vitro diagnostic reagent that should not be used for any other purposes.
2. Diagnosis should be made comprehensively based on other relevant test results and clinical symptoms .
3. Product has confirmed its usefulness as an ancillary test for evaluating the severity of atopic dermatitis in patients aged 0-15 years, so it should not be used for examining patients aged 16 years or older.
4. SCCA2 levels may also increase in malignancies, but there is no evidence that it is a tumor marker. Do not use this medicine to aid in the diagnosis of malignancies.
5. No assurance about how to use the product other than the package insert.
6. Read the package insert and instructions for use of the device carefully before use.
7. 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 [Abbreviation: SCCA Antibody]

(2) Standard

(3) Specimen Diluent

(4) POD-conjugate

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

(5) Conjugate Solvent

(6) Color Reagent A

3,3',5,5'-Tetramethylbenzidine dihydrochlorate dehydrate [Abbreviation: TMBZ]

(7) Color Reagent B

Hydrogen peroxide

(8) Stop Solution

(9) Wash Concentrate

(10) Control Sample

Accessory:Plate seal

【Intended use】

Measuring SCCA2 in Sera (an Aid for Assessing the Severity of Atopic Dermatitis in Children Aged 15 Years and Younger)

[Assay principle].

This kit utilizes an enzyme-linked immunosorbent assay (ELISA) method to quantify SCCA2 in serum. Samples are added to microtiter plate with 96-wells (solid phase) coated with anti-SCCA antibody, and after washing, a POD-conjugated anti-SCCA2 antibody is added to react, an immune complex is formed. After the unreacted POD-conjugated anti-SCCA2 antibody is removed by washing, a chromogenic substrate is added to perform the enzymatic reaction, and color develops in proportion to the amount of SCCA2 in the samples. The color development is stopped, and the color intensity is measured at 450nm.

SCCA2 concentration in the sample bound to the antibody in the solid phase is reflected in the chromogenic amount associated with the reaction between the POD and the chromogenic substrate, and therefore, SCCA2 concentration is determined by measuring this.

through SCCA2 in the specimen. and SCCA2 in the samples specifically binds to the antibody When SCCA2 in a sample is reacted in a 96-well microplate well (solid phase) in which an anti SCCA antibody is solidified, and after washing, a POD-labeled anti SCCA2 antibody is added to react, an immune complex is formed through SCCA2 in the specimen. After the unreacted POD-labeled anti SCCA2 antibody is removed by washing, a chromogenic substrate is added to perform the enzymatic reaction. SCCA2 concentration in the sample bound to the antibody in the solid phase is reflected in the chromogenic amount associated with the reaction between the POD and the chromogenic substrate, and therefore, SCCA2 concentration is determined by measuring this.

[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℃ 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 necessary for samples showing high measurement values.
3. Wear gloves and masks whenever handling specimens, reagents, equipment, etc.

Cross-reactivity

It does not react with SCCA1.

Interfering substances and drugs

1. Measurements are not affected up to 500 mg/dL Hb.
2. Measurements are not affected up to 50 mg/dL of bilirubin.
3. Measurements are not affected up to 500 IU/mL Rheumatoid Factor.
4. Measurements are not affected up to 3000 degrees of chyle (formazin turbidity).

[Operating method]

Preparation of reagents

1. Antibody-coated Plate
   (1) Use the antibody binding plate as it is.
2. Standard solution
   Prepare the (2) Standard to 0.2,0.1,0.05,0.025,0.0125, 0.0063 ng/mL with the (3) Specimen Diluent.
3. Specimen Diluent
   (3) Use the sample diluent as it is.
4. POD-conjugate solution
   Use (4) POD-conjugate by dissolving them in 1 bottle of 5) Conjugate Solvent. After dissolution, seal tightly and store at 2 to 8℃, it can be used for 2 weeks.

5. Chromogenic Reagent
   Mix equal volumes of (6)Color Reagent A and (7)Color Reagent B to form a chromogenic reagent. Use within 60 minutes at 20-30℃ storage after mixing.
6. Stop Solution
   (8) Use the reaction stop solution as it is.
7. Wash Solution
   Add 4 volumes of purified water to 1 volume of (9) Wash Concentrate, mix, and use as a wash solution.
8. Control Sample
   Use the (10) Control Sample as it is.

Necessary instruments, equipment, samples, etc.

1. Micropipette or Meth pipette
   (for reagent, sample preparation and dispensing)
2. Test tube (for sample diluent)
3. Vortex mixer
4. 1 L volumetric cylinder (for wash solution preparation)
5. Continuous dispenser (for dispensing reagent)
6. Micro plate washer (for washing)
7. Incubator (20°C to 30°C for reaction)
8. Microplate read

Measurement (manipulation) method

Take each reagent out of the refrigerator at least 30 minutes before use and return it to 20-30℃ before use.
Product is used in a variety of test instruments, so we illustrate an example of how to operate it.

1. Add 10 µL of serum to 1 mL of the specimen dilution and prepare the sample solution (101-fold dilution).
2. Dispense exactly 100 µL each of the specimen diluent (SCCA2 density: 0 ng/mL), prepared standard solution, sample solution and control sample, into the indicated wells of the antibody-binding plate. Measurements are taken in duplicate.
3. Incubate for 16-24 hours at 20-30°C. Cover the wells with a plate seal to prevent dust, dust, skin epidermis, saliva, perspiration, or other body fluids from getting into them during standing.
4. Aspirate and remove the reaction solution in the well with an aspirator.
5. Dispense 400 µL of wash solution into each well of the antibody binding plate. Aspirate and remove the dispensed wash solution with an aspirator, etc. Repeat this procedure five times.
6. Remove the remaining washing solution from the wells thoroughly by means of a paper towel, etc., and then perform the following operation.
7. Dispense 100 µL of POD-conjugate solution into each well of the antibody binding plate.
8. Cover the well with a plate seal and incubate for 90 minutes at 20-30°C.
9. Perform the same cleaning procedures as described in 4. to 6.
10. Dispense 100 µL of Chromogenic Reagent into each well of the antibody-binding plate.
11. Allow to stand for 30 minutes at 20-30°C. Cover with a plate cover or wrap, etc., to prevent dust and dirt from entering during standing.
12. Dispense 100 µL of Stop Solution into each well of the antibody binding plate.
13. Measure absorbances of 450 nm (main wavelength) and 550-700 nm (secondary wavelength) for all wells. Measurements should be performed within 120 minutes in an environment between 20°C and 30°C after cessation of the reaction.

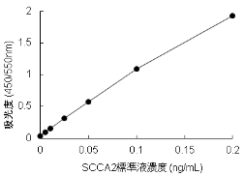
Outline of measurement procedure

Preparation of the sample solution (diluted 101-fold serum)
↓
Dispense the prepared standard solution, sample solution, and control sample
(100 µL/well each).
↓ 20-30°C for 16-24 h [primary reaction]
Wash (5 times)
↓
Dispense POD-conjugate solution (100 µL/well)
↓ 20-30℃ for 90 minutes [secondary reaction]
Wash (5 times)
↓
Dispense chromogenic reagent (100 µL /well)
↓ 20-30℃, 30 minutes [chromogenic reaction]
Dispense stop solution (100 µL/well)
↓
Determination of absorbance, main wavelength (450 nm) and secondary wavelength (550-700 nm).

Calculation of measured results

1. Subtract the absorbance of the secondary wavelength (550-700 nm) from the absorbance of the major wavelength (450 nm).
2. Plot the concentration and absorbance of each standard solution to create a standard curve.
3. Calculate SCCA2 concentration (ng/mL) of the sample solution from the standard-curve.
4. Calculate the serum SCCA2 level by multiplying the concentration of the specimen solution obtained by the diluent fold of the sample.
5. The average value of each concentration measured in duplicate for each sample is used as a measurement value.
6. Specimens that exceed the measurement range should be appropriately diluted with the sample diluent and re-measured.

Standard curve example



[Decision method of measurement results]

Reference standard range

In the clinical performance test results of product, the serum SCCA2 levels of 159 non-allergic patients aged 0 to 15 years and 176 patients with atopic dermatitis were tabulated and analyzed. Serum SCCA2 levels in non-allergic individuals did not differ significantly by sex, but did differ significantly by age. The cut-off for serum SCCA2 level obtained from ROC-analysis in all patients was 1.6 ng/mL.
The 95th percentile for non-allergic individuals was also 1.6 ng/mL. The reference range for 0-15-year-olds was set to less than 1.6 ng/mL because the baseline value tended to be higher than 1.6 ng/mL for 0-year-olds and lower for 6-year-olds or older, but this clinical performance test was difficult to conclude clearly due to the limited number of age groups.

Criteria for severity assessment

Based on the ROC-analysis of mild (n=56), moderate (n=60), and severe (n=60) serum SCCA2 levels in patients with atopic dermatitis, mild disease was defined as less than 1.6 to 2.6 ng/mL, moderate disease as 2.6 to 6.0 ng/mL, and severe disease as 6.0 ng/mL or more. Objective-SCORAD (O-SCORAD) was used as an index of the severity of atopic skin irritation, and scores of less than 15 were classified as minor, 15 or more than 40 as moderate, and 40 or more as severe.
However, these values may fluctuate under various conditions, such as the number of subjects in the target group and the age group. Therefore, the doctor should comprehensively determine the serum SCCA2 level based primarily on the observation/assessment of individual skin rashes in order to evaluate the diagnosis and severity of atopic dermatitis.

Reference standard range and criteria for severity

	Serum SCCA2 value
Reference standard range	<1.6 ng/mL
Mild disease	≥1.6 ng/ mL and< 2.6 ng/mL
Moderate disease	≥2.6 ng /mL and < 6.0 ng/mL
severe	6.0 ≥ ng/mL

[Clinical significance]

SCCA(squamous cell carcinoma antigen) is a serine protease inhibitor belonging to serpin superfamily, which is a protein with a molecular weight of about 45 kDa produced mainly by epithelial cells.

SCCA contains the isoforms SCCA1(SERPIN B3) and SCCA2(SERPIN B4, which are independent gene products transcribed from distinct loci, albeit with very high-amino-acid homologies of 91%.It is known that interleukin 13 (IL-13), one of the type Th2 cytokines, plays crucial roles in allergic inflammation. SCCA2 was identified as one of the molecules whose expression is elevated when airway epithelial cells are stimulated with IL-4 and IL-13, cytokines that are central in allergic responses[1]. Serum SCCA2 levels in children with atopic dermatitis have been reported to be significantly elevated according to the severity of the disease, even when compared with existing blood tests [2,3,4]. In the treatment of atopic dermatitis, it is important to select and use anti-inflammatory drugs appropriately according to their severity. It was suggested that serum SCCA2 could be a useful marker as an indicator of the severity of childhood atopic dermatitis. Clinical performance test results of product in 159 allergic unaffected patients and 176 patients with atopic dermatitis aged from 0 to 15 years are shown below.

1. Comparison of serum test values by severity of atopic dermatitis.

Serum SCCA2 levels were significantly higher depending on the severity of atopic dermatitis. On the other hand, the serum TARC and total IgE levels of the control test items were generally high depending on the severity, but did not differ significantly between mild and moderate disease (Fig. 1).

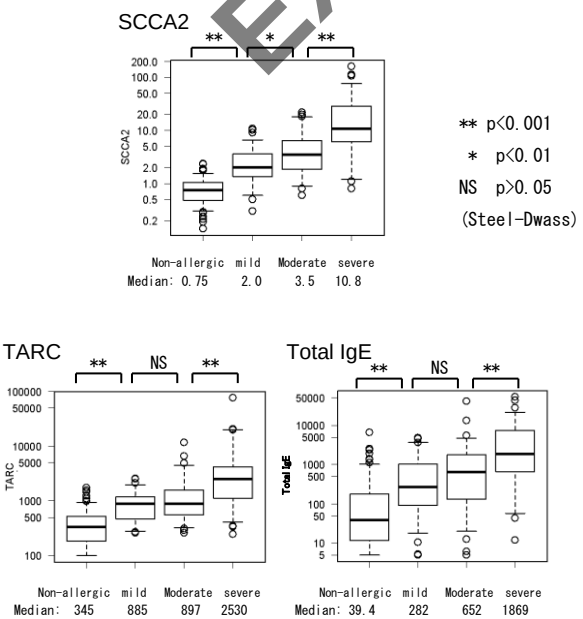


Figure 1. Serology by severity

2. Comparison between patient group and non-allergic group

(1) We performed ROC analysis of serum test values in patients with atopic dermatitis and all allergic unaffected patients, and compared the areas under the ROC curves (AUCs: area under the curve) of each of them. The AUCs for SCCA2, TARC and IgE were 0.929, 0.871, and 0.822, respectively, which were significantly different (Fig. 2).
At this time, the cut-off level for serum SCCA2 (the highest sensitivity + specificity) was 1.6 ng/mL, and the sensitivity (the prevalence rate of correct diagnosis) and specificity (the disease-free rate) were 80% and 95%, respectively.

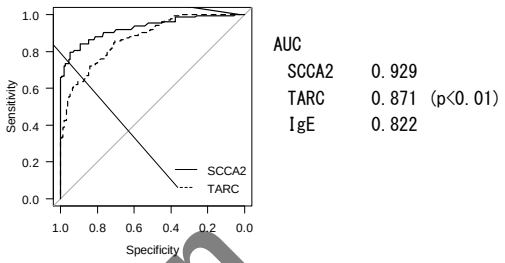


Figure 2 ROC analysis in atopic dermatitis patients and allergic unaffected individuals.

(2) Since there was a significant age-related difference in allergic unaffected patients, ROC analysis was performed for each age group. Serum SCCA2 cutoff (sensitivity + specificity maximized) and sensitivity and specificity at that time are shown (Table 1). The AUCs of SCCA2 and TARC were higher in all age groups, but there were no significant differences due to the small sample size for each age group. Therefore, ROC analysis was performed by dividing each age into three groups (0-1, 2-6, and 7-15 years) (Figure 3). Although there were no significant differences from TARC in those aged 7 years or older, those aged 6 years or younger had significantly higher AUCs for SCCA2.

Table 1 ROC-analysis of SCCA2 by age-group.

Age	N (non-patient/patient)	Cut off value	sensitivity	Specificity	AUC
0	10/26	2.0	0.96	1.00	0.989
1	22/17	1.6	0.94	1.00	0.992
2	19/22	1.4	0.96	1.00	0.923
3	21/17	1.6	0.77	1.00	0.871
4	17/18	1.3	0.89	0.94	0.971
5	19/19	1.4	0.79	0.95	0.884
6	10/16	1.0	0.94	1.00	0.963
7-15	41/41	1.0	0.90	0.85	0.948
0-1	32/43	2.0	0.91	1.00	0.988
2-6	86/92	1.4	0.82	0.93	0.906
0-15	159/176	1.6	0.80	0.95	0.929

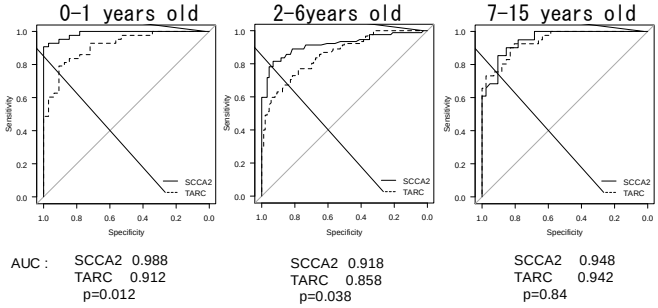


Fig 3 ROC analysis of each age divided into three groups

3. Progress case

For the follow-up cases (n=31), serum SCCA2 levels also decreased over the course of treatment (Fig. 4) and were significantly correlated with changes in O-SCORAD (Fig. 5).

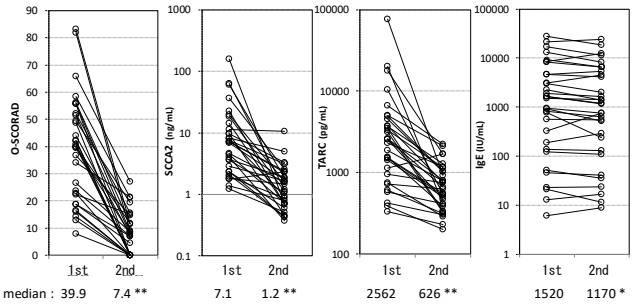


Figure 4. Treatment course changes

** p<0.001 * p<0.05
(Wilcoxon's signed rank test)

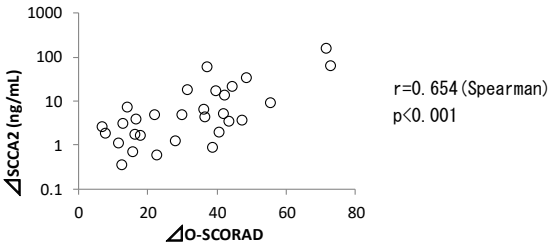


Figure 5. Correlation of treatment course variability values

[Performance]

Performance

1. Sensitivity, Accuracy, Simultaneous Reproducibility
   (1) Sensitivity
   The absorbance of the standard solution is greater at higher SCCA2 concentrations, and the absorbance difference between the 0 ng/mL standard solution and the 0.2 ng/mL standard solution is 1.20 or higher.
   (2) Accuracy
   When a control sample with a known concentration is measured, accuracy is within ±20% of the known SCCA2 concentration is 1.0 ng/mL or higher, and within ±30% of the known SCCA2 concentration is less than 1.0 ng/mL.
   (3) Simultaneous Reproducibility
   When the same sample is measured three times simultaneously, the C.V. value of the measured 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, etc. 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 shown before if you wish to receive SDS.

Usage

1. Avoid freezing the kit. Follow storage instruction. Please do not use the kit if it has been frozen, as the results obtained may not be accurate.
2. Do not use the kit after the expiration date.
3. Do not use the kit with different manufacturing numbers in combination. Also, do not pour over reagents even if they are reagents of the same manufacturing number.
4. Crystals may be found in the mouth of bottles of wash concentrated, but their performance is not problematic when used as such.
5. Do not reuse the container or diverse it for other purposes.

Disposal

1. Specimen may contain HIV, HBV, HCV and other pathogens. After use, waste and equipment must be decontaminated by soaking in sodium hypochlorite (1,000 ppm chlorine, 1 hour or longer) or glutaraldehyde (2%, 1 hour or longer.) or sterilized by autoclave (121°C, 20 minutes or longer.).
2. If specimens or solutions containing specimens are spilled wipe the surface with 80% alcohol spray, etc.
3. The specimen diluent contains trace amounts of sodium azide. Sodium azide may form explosive metals azide when in contact with lead or copper. For safety, dilute with water plenty of water when disposing of Specimen Diluent or reaction waste.
4. Reagents and equipment must be appropriately disposed of by observing local regulations.

[Storage method and shelf life]

12 months at 2–8 ºC.

[Packaging unit]

Uniform product code Product name	Package
326076529	
IMCURE SCCA2	48 times
(1) Antibody-coated Plate	1 pcs
(2) Standard	1 mL × 1
(3) Specimen Diluent	100 mL × 1
(4) POD-conjugate	(12.0 mL) x 1
(5) Conjugate Solvent	12 mL × 1
(6) Color Reagent A	6 mL × 1
(7) Color Reagent B	6 mL × 1
(8) Stop Solution	12 mL × 1
(9) Wash Concentrate	100 mL × 2
(10) Control Sample	1 mL × 1
Accessories: Plate seal 2 pcs	

[References]

1. Yuyama N., et al. : Cytokine ; 19: 287-296（2002）
2. Ohta S., et al. : Ann. Clin. Biochem. ; 49: 277-284（2012）
3. Nagao M., et al. : J. Allergy Clin. Immunol. ; 141: 1934-1936（2018）
4. T. Fujisawa : Allergy; 67: 981-986 (2018)

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