RIDASCREEN® sIgA

REF G09035

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1. Intended use

For *in-vitro* diagnostic use. RIDASCREEN® sIgA is an enzyme immunoassay for the quantitative detection of human secretory IgA (sIgA) in stool samples.

2. Summary and explanation of the test

IgA is the most heterogeneous of all human immunoglobulins. IgA antibodies are present in external body fluids and form a major barrier of defense against pathogens.
Fecal concentrations of sIgA can provide information on the autoimmune status of the intestinal mucosae.
IgA occurs in a monomeric (mIgA), polymeric (pIgA) and dimeric secretory form (sIgA). The formation of secretory IgA is independent of serum IgA synthesis. Secretory IgA consists of two (monomeric) IgA molecules, a J chain, a secretory component (SC chain), and a polypeptide with a molecular mass of 70 kDa. The secretory component is synthesized by the epithelial cells of the mucosae of the gastrointestinal, respiratory, and urogenital tracts and of the salivary, lacrimal, and mammary glands. The plasma cells in the subendothelial space of the mucosae secrete a complex consisting of two IgA molecules linked to each other via the J protein. This complex then binds to the secretory component, which is located on the surface of the epithelial cell. After binding, the sIgA is selectively transported through the epithelial cell by means of epithelial receptors and excreted on the surface of the mucosae by exocytosis. In this way, secretory IgA (sIgA) is transported in large quantities, e.g., to the surface of the intestinal mucosae. Secretory IgA can, however, also be found in other body secretions such as mother's milk, saliva, tears, and nasal and tracheobronchial mucus.
The determination of sIgA concentrations in stool can provide information about the functional status of gut-associated lymphoid tissue (GALT), which is the immune system of the intestine. sIgA is an indicator of the secretory performance and stimulation level of plasma cells in the intestinal submucosa.
A sIgA deficiency is reflective of decreased intestinal mucosal immune defense activity, whereas increased sIgA levels indicate that activity of the intestinal immune system is increased.
Considering the strong anti-inflammatory properties of IgA, increased fecal concentrations of sIgA suggest the presence of local inflammatory responses in the intestinal mucosa.

RIDASCREEN® sIgA for rapid and reliable detection of:
- Functional status of the gut-associated lymphoid tissue (GALT)
- Decreased sIgA level: reduced activity of the gut-associated immune system
- Increased sIgA level: increased activity of the gut-associated immune system
- Impaired immunological barrier function of the intestinal mucosa (increased susceptibility to infection, allergic disease)
- Local inflammation of the intestinal mucosa
- Autoimmune diseases

3. Test principle

In RIDASCREEN® sIgA, specific antibodies are used in a sandwich-type method. The surface of the well of the microtiter plate is coated with a specific antibody against epitopes of human sIgA. A suspension of the stool sample to be tested is pipetted into a well of the microtiter plate and incubated. This is followed by a washing step and a second incubation together with a monoclonal antibody that is conjugated with horseradish peroxidase. In the presence of sIgA, the immobilized antibody, sIgA antigen, and conjugated antibody form a sandwich complex. Unbound enzyme-marked antibodies are removed in a subsequent washing step. In positive samples, after the addition of a substrate, the bound enzyme changes the colorless solution in the wells of the microtiter plate to a blue solution. The addition of a stop solution changes the color from blue to yellow. The measured absorbance is proportional to the sIgA concentration present in the sample.

4. Reagents provided

The reagents in the kit are sufficient for 96 determinations.

Plate	96 assays	Microtiter plate, 12 microtiter strips (breakable) in a strip holder; coated with polyclonal antibodies (from rabbits) against secretory IgA
Extract 10x	100 ml	Extraction and dilution buffer (10x conc.); for extraction and primary dilution of stool samples; phosphate-buffered NaCl solution; contains 0.1 % Tween und 0.1 % NaN_3
Diluent | 3	100 ml	Sample dilution buffer 3 (for final dilution), protein-buffered NaCl solution; contains 0.1 % NaN_3; ready for use, dyed red
Wash buffer	100 ml	Wash buffer 10x (10x concentration); phosphate-buffered NaCl solution; contains 0.1 % thimerosal
Calibrator	1 ml	Calibrator (for standard calibration); contains 0.1 % NaN_3; ready for use
High control	1 ml	High control; contains 0.1 % NaN_3; ready for use
Low control	1 ml	Low control; contains 0.1 % NaN_3; ready for use
Conjugate	12 ml	Peroxidase conjugated, monoclonal antibody (mouse) against human sIgA in stabilized protein solution; ready for use
SeroSC	12 ml	Substrate; hydrogen peroxide/TMB; ready for use
Stop	12 ml	Stop solution; 1 N sulfuric acid; ready for use

Dangerous substances are indicated according to labeling obligations. For further details, please refer to the safety data sheets (SDS) at www.r-biopharm.com.

5. Storage instructions

All reagents must be stored at 2 - 8 °C and can be used until the expiry date printed on the respective label. The diluted wash buffer and the diluted extraction buffer can be used for a maximum of 4 weeks when stored at 2 - 8 °C. Microbial contamination must be prevented. After the expiration date, the quality guarantee is no longer valid. Use scissors to open the aluminum bag containing the microtiter strips, but take care to not tear off the clip seal. Any microtiter strips that are not required must immediately be sealed in the aluminum bag and stored at 2 - 8 °C. The colorless

substrate must also be protected from direct light to prevent it from decomposing or turning blue due to auto-oxidation. Once the substrate has turned blue, it must not be used.

6. Additional necessary reagents and necessary equipment

6.1. Reagents

- Distilled or deionized water

6.2. Equipment

- Microbalance
- Vortex mixer (optional, see 9.4.)
- Micropipette for 50 - 100 µl and 1 ml volumes
- Graduated cylinder (1,000 ml)
- Stopwatch
- Washing device for microtiter plates or multichannel pipettes (300 µl)
- Microtiter plate photometer (450 nm; reference filter ≥ 620 nm)
- Filter paper (lab wipes)
- Waste container containing 0.5 % hypochlorite solution

7. Warnings and precautions for the users

For *in-vitro* diagnostic use only.
This test must be carried out only by trained laboratory personnel. The guidelines for working in medical laboratories must be followed. Always adhere strictly to the user instructions for carrying out this test. Do not pipette samples or reagents using your mouth. Avoid contact with broken skin or mucous membranes. Wear personal protective equipment (appropriate gloves, lab coat, safety glasses) when handling reagents and samples, and wash hands after completing the test. Do not smoke, eat, or drink in areas where samples are handled.
For further details, see the safety data sheets (SDS) at www.r-biopharm.com.
The stool samples should be handled as if potentially infectious, pursuant to the national safety requirements.
The wash buffer contains 0.1 % thimerosal as preservative. This substance must not be allowed to come into contact with skin or mucous membranes.
Calibrator, high control, low control, extraction and sample dilution buffer contain 0.1 % NaN_3 as a preservative. This substance must not be allowed to come into contact with skin or mucous membranes.
Hydrogen peroxide (substrate) can cause chemical burns. Handle with care.
The Stop solution contains 1 N sulphuric acid. Avoid contact with the skin and clothing. If the reagent comes into contact with your skin, rinse your skin off with water.

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121 °C for at least one hour.
CAUTION: To prevent the formation of toxic gases, any liquid waste containing stop reagent must be neutralized before adding to hypochlorite solution.
Users are responsible for proper disposal of all reagents and materials after use. For disposal, please adhere to national regulations.

8. Collection and storage of samples

The stool samples should be transported chilled if possible and stored at 2 - 8 °C before testing. We recommend immediate preparation of the sample or storage at -20 °C or colder. Avoid freezing and thawing the sample repeatedly. Stool samples should not be collected in transport containers that contain transport media with preservatives, animal sera, metal ions, oxidizing agents, or detergents since these may interfere with the RIDASCREEN® sIgA test.

9. Test procedure

9.1. General information

All reagents and the microtiter plate Plate must be brought to room temperature (20 - 25°C) before use. Once they have reached room temperature, remove the microtiter strips from the aluminum bag. Mix the reagents well immediately before use. After use, the unused microtiter strips (placed in sealed bags) and the reagents must be stored again at 2 - 8°C. Once used, the microtiter strips must not be used again. Do not use reagents or microtiter strips if the packaging is damaged or the containers are not tightly sealed. In order to prevent cross-contamination, the samples must be prevented from coming into direct contact with the kit components. The test must not be carried out in direct sunlight. We recommend covering the microtiter plate or sealing with plastic wrap to prevent evaporation losses.

9.2. Preparing the wash buffer

Mix 1 part wash buffer concentrate Wash 10x with 9 parts distilled water (1:10). For this step, add 100 ml concentrate to a 1,000 ml measuring cylinder and fill with distilled water to 1,000 ml. Any crystals present in the concentrate should be dissolved beforehand through heating (water bath at 37 °C).

9.3. Preparing the extraction buffer

Mix 1 part extraction buffer concentrate Extract 10x with 9 parts distilled water (1:10). For this step, add 100 ml concentrate to a 1,000 ml measuring cylinder and fill with distilled water to 1,000 ml. Any crystals present in the concentrate should be dissolved beforehand through heating (water bath at 37 °C).

9.4. Sample preparation

9.4.1. Sample weighing and suspension

We recommend storing the stool samples at -20 °C until preparation. Frozen samples should be slowly brought to room temperature.
Weigh 100 mg of stool sample into a labeled test tube, and then add 5 ml of the diluted extraction buffer using a pipette (1:50 dilution).
Alternatively, between 80 and 130 mg of the stool sample is weighed out, and to ensure a constant dilution ratio (1:50), the volume of the diluted extraction buffer used varies depending on the weight of the stool sample (see Table 1).

Table 1: Data on the required amounts of diluted extraction buffer depending on the amount of stool weighed in (constant dilution factor of 1:50)

Sample weight [mg]	Volume [ml]
80	4.00
85	4.25
90	4.50
95	4.75
100	5.00
105	5.25
110	5.50
115	5.75
120	6.00
125	6.25
130	6.50

During re-suspension in a constant extraction buffer volume of 5 ml, the variable dilution factor must be taken into consideration in the calculation (see Table 2).

Table 2: Data on the dilution factor with constant addition of diluted extraction buffer (5 ml), depending on the weight of the stool

Sample weight [mg]	Dilution factor
80	62.50
85	58.82
90	55.55
95	52.63
100	50.00
105	47.62
110	45.45
115	43.45
120	41.66
125	40.05
130	38.46

The stool suspension is homogenized by thoroughly mixing it on a vortex mixer, regardless of the method used to weigh the stool sample. If the stool is in liquid form, use a pipette to draw exactly 100 µl and suspend it in exactly 5 ml diluted extraction buffer.

Then the homogenized suspension must be centrifuged for 10 minutes at least 3,000 g to settle out rough stool particles. The supernatant of the extract must be further diluted immediately for use in the assay. We recommend storing the aliquoted supernatant at -20 °C or colder.

9.4.2. Manual sample dilution

Dilute 50 µl of the clarified supernatant (see 9.4.1) in 950 µl diluted extraction buffer (1:20) and vortex the sample. The sample is then further diluted by diluting 100 µl of the first dilution with 900 µl of RIDASCREEN® sample dilution buffer Diluent | 3 (1:10), followed by vortexing. This final dilution of the stool sample (1:10,000) will be used in the test.
A suitable alternative method for the processing and homogenization of the sample material described above is the sample-preparation system supplied by the company. R-Biopharm AG will gladly provide special instructions on how to use this method on request.

9.4.3. Automatic sample dilution

If the assay is to be run on the DSX automated ELISA system (Dynex Technologies, Inc.), the specific assay protocol required for this should be requested from R-Biopharm AG and applied to the system. The sample is then automatically diluted as described below.

25 µl of the clarified supernatant is pipetted by the automatic ELISA system into a deep-well plate and diluted with 975 µl of diluted extraction buffer (1:40). Two mixing cycles follow.
The required number of microtiter strips is placed in the strip holder for the RIDASCREEN® sIgA microtiter plate Plate.
Individual 20 µl volumes of the sample dilution are transferred from the deep-well plate to the RIDASCREEN® sIgA microtiter plate Plate and diluted further with 80 µl of RIDASCREEN® sample dilution buffer Diluent | 3 (1:5; final dilution 1:10,000).
For instructions on how to perform the assay with other ELISA automated pipetting systems, please contact R-Biopharm AG.

9.5. First incubation

After placing a sufficient number of wells in the holder, add 100 µl of calibrator Calibrator (in duplicate), RIDASCREEN® sample dilution buffer Diluent | 3 (= negative control), high control High control | + and the final stool sample dilutions being analyzed to the relevant wells. If used, add 100 µl of the low control Low control | + to the test. Next, incubate the plate for one hour at room temperature (20 - 25°C).

9.6. First washing

Careful washing is important in order to achieve the correct results and should therefore be carried out strictly in accordance with the instructions. The incubated substance in the wells must be emptied into a waste container containing the hypochlorite solution for disinfection. Next, tap the plate over absorbent paper to remove the remaining moisture. Then wash the plate 5 times using 300 µl diluted wash buffer each time (see 9.2). Make sure that the wells are emptied completely by tapping them out after each wash onto a dry and unused area of absorbent paper. When using a microplate washer, make sure that the machine is correctly adjusted to the type of microtiter plate being used. Furthermore, a stool suspension that is not completely particle-free before the first wash should be removed from the cavities manually by centrifuging in order to avoid clogging the wash needles. Also ensure that all of the liquid is aspirated during each washing stage. After washing for the last time, knock out the plate thoroughly onto clean absorbent paper or laboratory towels in order to remove any residual moisture.

9.7. Second incubation

Add 100 µl conjugate Conjugate to each well. Next, incubate the plate for one hour at room temperature (20 - 25°C).

9.8. Second washing

Following the incubation period, empty the conjugate in the wells into a waste container containing hypochlorite solution for disinfection. Next, tap the plate over absorbent paper to remove the remaining moisture. Then wash the plate 5 times using 300 µl wash buffer each time. Make sure that the wells are completely empty by tapping them out after each wash onto a dry and unused area of absorbent paper.

9.9. Third incubation

Add 100 µl substrate SeroSC to each well. Then incubate the plate for 15 minutes in darkness at room temperature (20 - 25°C). Subsequently, stop the reaction by adding 50 µl Stop solution Stop to each well.
After mixing carefully (by tapping lightly on the side of the plate), measure the absorbance at 450 nm and at a reference wavelength of 620 nm.

Note: High-positive patient samples may cause black-colored precipitates of the substrate. These samples should be diluted 1:10 and used in the test again (see 9.5.) (final dilution of the stool sample: 1:10,0000).

10. Quality control — indication of instability or deterioration of reagents

For quality control purposes, the calibrator Calibrator (in duplicate), the RIDASCREEN® sample dilution buffer Diluent | 3 as a negative control, and the high control High control | + must be used every time the test is carried out to ensure the stability of the reagents and correct performance of the test. The use of the low control is optional.

The test has been performed correctly when the extinction mean (OD) of the negative control at 450 nm/620 nm is less than 0.05, and the averaged extinction mean (OD) of the calibrator is within the range indicated on the batch-specific data sheet. The high control must lie within the batch-specific concentration range on the data sheet.

When RIDASCREEN® sIgA is processed on open, fully automated ELISA systems, the measured OD of the calibrator Calibrator may deviate from the range indicated on the batch-specific certificate depending on the system. Even on fully automated ELISA systems, the high control High control | + is decisive for the validity of the test results and must therefore always be tested. It is not absolutely essential to use the low control Low control | +.

Deviation from the required values, reagent turbidity, or blue coloring of the colorless substrate before adding to the wells may indicate that the reagents have expired. If

the stipulated values are not met, the following points must be checked before repeating the test:

- Expiration date of the reagents used
- Functional performance of the equipment used (e.g., calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks; a substrate solution that has turned blue must not be used.

If the conditions are still not met after repeating the test, please consult the manufacturer or your local R-Biopharm distributor.

11. Evaluation and interpretation

11.1. Single-point quantification according to the 4-parameter logistic log model

RIDASCREEN® sIgA uses the 4-parameter logistic log model (4PL) to determine the concentration of sIgA in a stool sample in µg/g.
The RIDA®SOFT Win.net evaluation software is required to calculate the results. RIDA®SOFT Win.net or updates can be obtained on request by contacting R-Biopharm AG or your local R-Biopharm distributor.
The parameters (A - D) required for the 4PL calculation of the standard curve and the target value for the calibrator, high control, and low control can be found on the batch-specific data sheet that comes with the test kit, and they must be compared with the values in the evaluation software before measurement.
In the final quality control, R-Biopharm AG calculates the standard curve (including parameters A - D) under optimum test conditions for each kit batch, as well as a target value and a permissible range for the standard deviation for the calibrator, high control, and low control.
The calibrator is also tested to compensate for test fluctuations and to check the quality of the test procedure. The high control provides information on the validity of the test.
RIDA®SOFT Win.net calculates correction factor F internally from the mean of the calibrator duplicate analysis and its target value. This correction factor is then reconciled with the absorbances for the stool samples. The test results can be confidently and reliably evaluated within the limits of the standard curve.
Other evaluation software that uses the 4-parameter logistic log model can also be used instead of RIDA®SOFT Win.net.

11.2. Test result

The normal range of sIgA is 100 - 1200 µg sIgA/g stool. If the results are below 100 µg sIgA/g stool, patients have insufficient sIgA levels. If the results are above 1200 µg sIgA/g stool, patients have elevated sIgA levels.
We advise each laboratory to establish its own standard value range.

12. Limitations of the method

The RIDASCREEN® sIgA kit detects epitopes of human sIgA in stool samples. A correlation between the level of the determined extinction mean with the severity of clinical symptoms cannot be derived from such. The obtained results must always be interpreted in connection with the clinical signs and symptoms.

13. Performance characteristics

13.1. Analytical sensitivity

The analytical sensitivity was determined by analyzing the limit of blank (LoB) using a total of 210 measurements of a buffer, consisting of an extraction buffer and diluent, and by analyzing the limit of detection (LoD) using 70 measurements of a positive control sample. The results are shown in Table 3.

Table 3: Results of the analytical sensitivity

	MV [OD 450/620 nm]	µg/g
LoB	0.003	-
LoD	-	65.07

13.2. Precision

The intra- and inter-assay reproducibility of RIDASCREEN® sIgA was determined multiple times on different days while maintaining optimal conditions. The mean value (MV), standard deviation (SD) and coefficient of variation (CV) were calculated from the results. Tables 3 and 4 show the results.

Tab.4: Intra-assay reproducibility (n = 16)

	1. Concentration [600 µg/g]	2. Concentration [1200 µg/g]
MV (ng/ml)	674	1380
SD	45.6	55.80
% CV	**6.8**	**4.0**

Table 5: Inter-assay reproducibility (n = 8)

	1. Concentration [660 µg/g]	2. Concentration [1500 µg/g]
MV (ng/ml)	677	1461
SD	79.7	85.5
% CV	**11.8**	**5.9**

13.3. Linearity of test results

To check the linearity of RIDASCREEN® sIgA, serial dilution series of several sIgA-containing stool samples were prepared. sample weights and suspension as well as the first sample dilution were determined as directed in these instructions (see 9.4). The serial dilution steps were then prepared using RIDASCREEN® sample dilution buffer Diluent | 3.

The concentrations determined in the test compared to those expected were calculated. Table 5 shows the dilution series of three samples with varying sIgA concentrations.

Table 5: Determination of linearity of the test results

Sample	Dilution	Determined concentration (µg/g)	Expected concentration (µg/g)	Determined conc. / expected conc.
1	1:10,000	807.00		
	1:20,000	406.00	403.50	101 %
	1:40,000	207.50	201.75	103 %
	1:80,000	104.25	100.88	103 %
	1:160,000	57.69	50.44	114 %
	MV			105 %
2	1:10,000	1175.00		
	1:20,000	576.50	587.50	98 %
	1:40,000	280.25	293.75	95 %
	1:80,000	146.38	146.88	100 %
	1:160,000	74.69	73.44	102 %
	MV			99 %
3	1:10,000	378.00		
	1:20,000	202.50	189.00	107 %
	1:40,000	107.25	94.50	113 %
	1:80,000	58.75	47.25	124 %
	1:160,000	-	23.63	-
	MV			115 %
	MV (total)			106 %

14. Version history

Version number	Chapter and designation
2019-02-27	General revision 14. Version history 15. Explanation of symbols

15. Explanation of symbols

General symbols

IVD	For in vitro diagnostic use
	Consult instructions for use
LOT	Lot number
	Expires
	Store at
REF	Article number
	Number of tests
	Date of manufacture
	Manufacturer

Test-specific symbols

Plate	Microtiter plate
Extract 10x	Extraction and dilution buffer
Diluent | 3	Sample dilution buffer
Wash buffer	Wash buffer 10x
Calibrator	Calibrator
High control	High control
Low control	Low control
Conjugate	Conjugate
SeroSC	Substrate
Stop	Stop solution

16. References

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