RIDASCREEN® Spec. IgG

Art. No.: A0020 RIDASCREEN® Spec. IgG Reagents

Art. No.: A0021 RIDASCREEN® Spec. IgG Reagents

applies also to:

Art. No.: A0049 RIDASCREEN® Allergen Disc

Art. No.: A0629 RIDASCREEN® Spec. IgG Allergen Disc

Art. No.: A0630 RIDASCREEN® Spec. IgG Allergy Panel, Customized

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1. Intended use

For *in vitro* diagnostic use. The RIDASCREEN® Spec. IgG Test is an enzyme immunoassay (EIA) for the quantitative determination of specific IgG antibodies in human serum and should be used in cases of suspected Type III allergy. However, the test can also be used for the follow up of hyposensitisation to hymenoptera toxins etc. This test is not suitable for HSA allergens.

2. Summary and explanation of the test

Type III allergy and its trigger can be determined by the detection of specific IgG antibodies. Type III allergies are characterised by the formation of immune complexes formed from free allergens and the specific IgG antibodies. These immune complexes can also be formed by external allergens on body surfaces such as the lungs, and lead to the clinical picture of exogenetic allergic alveolitis. Farmer's lung or pigeon breeder's disease is caused by constant exposure to fungus from mouldy hay or pigeon antigens.

These immune complexes can also develop as a result of food or food components penetrating through the wall of the intestine, depositing in the tissue and causing inflammation reactions, thus leading to a variety of clinical pictures.

The determination of IgG antibodies is also used for therapeutic monitoring in the case of allergies to hymenoptera toxins.

The test is a cellulose-disc-based enzyme allergo-sorbent test (EAST). All reagents (A0021) listed under Section 4 are validated using the allergen discs (A0029) from R-Biopharm. When using these reagents with discs from other suppliers, it is the duty of the user to carry out a validation test.

3. Test principle

The allergens are attached to the cellulose discs. In order to carry out the test, the allergen discs are placed in the wells of a microwell plate or are pre-coated according to the requirements of the doctor.

The allergen discs for the allergy test (Art. No. A0630) are already inserted in the wells of 16-microwell strips. Five 16-microwell strips from different kits can be combined on one frame to yield one full plate. However, Columns A and B must always be used for standards and controls

Patient sera, standard sera and negative and positive controls are pipetted onto the allergen discs and incubated at 37°C. During incubation, the allergy-specific IgG antibodies attach themselves to the allergen. Material which does not attach is removed by washing. After this, an anti-human IgG antibody, which is conjugated with alkaline phosphatase, is also added. Any conjugate which is not attached is removed by washing. Substrate is then added and is dephosphorylated by the conjugated enzyme into a yellow product. The intensity of the yellow colour is proportional to the quantity of allergen-specific IgG antibodies in the serum.

A photometric measurement is carried out at 405 nm using a reference wavelength of 620 nm.

4. Reagents provided

Table1: Pack content of RIDASCREEN® Spec. IgG Reagents (Article no. A0021)

Plate	1-96 det.	1 microwell plate
DiscS	8	Standard discs
DiscC	4	Control discs
DiscA		Allergen discs, number according to customer's requirements (not included in the reagent kit A0021)
Diluent	120 ml	Sample dilution buffer, ready for use
AllergyWB	60 ml	Wash buffer, x 16 concentrate, 0,9% NaN_3 Lid colour: transparent
Standard 1	250 µl	Standard serum 1, dil. human serum; concentration (in µg IgG/ml): 2.5 ; in stabilised protein solution
Standard 2	250 µl	Standard serum 2, dil. human serum; concentration (in µg IgG/ml): 10 ; in stabilised protein solution
Standard 3	250 µl	Standard serum 3, dil. human serum; concentration (in µg IgG/ml): 40 ; in stabilised protein solution
Standard 4	250 µl	Standard serum 4, dil. human serum; concentration (in µg IgG/ml): 200 ; in stabilised protein solution
Control high	250 µl	High Control IgG, human serum, ready for use, concentration: see certificate; in stabilised protein solution

Control low	250 µl	Low Control IgG, human serum, ready for use, concentration: see enclosed certificate; in stabilised protein solution
Conjugate	6 ml	Anti-human IgG-conjugate, (goat); ready for use; alkali phosphatase conj. antibodies in stabilised protein solution, Lid colour: white
AllergySub S	12 ml	Substrate, pNPP solution, ready for use Lid colour: brown
AllergyStop R	12 ml	Stop Reagent, ready for use Lid colour: green

Table 2: Pack contentof RIDASCREEN® Spec. IgG Reagents (Article no. A0020)

Plate	1-192 det.	2 microwell plates
DiscS	16	Standard discs
DiscC	8	Control discs
DiscA		Allergen discs, number according to customer's requirements (not included in the reagent kit A0020)
Diluent	2 x 120 ml	Sample dilution buffer, ready for use
AllergyWB	2 x 60 ml	Wash buffer, x 16 concentrate, 0,9% NaN3
Standard 1	250 µl	Standard serum 1, dil. human serum; concentration (in µg IgG/ml): 2.5 ; in stabilised protein solution
Standard 2	250 µl	Standard serum 2, dil. human serum; concentration (in µg IgG/ml): 10 ; in stabilised protein solution
Standard 3	250 µl	Standard serum 3, dil. human serum; concentration (in µg IgG/ml): 40 ; in stabilised protein solution
Standard 4	250 µl	Standard serum 4, dil. human serum; concentration (in µg IgG/ml): 200 ; in stabilised protein solution
Control high	250 µl	High Control IgG, human serum, ready for use, concentration: see certificate; in stabilised protein solution

Control low	250 µl	Low Control IgG, human serum, ready for use, concentration: see certificate; in stabilised protein solution,
Conjugate	12 ml	Anti-human IgG-conjugate, (goat); ready for use; alkali phosphatase conj. antibodies in stabilised protein solution, Lid colour: white
AllergySub S	2 x 12 ml	Substrate, pNPP solution, ready for use Lid colour: brown
AllergyStop R	2 x 12 ml	Stop Reagent, ready for use Lid colour: green

5. Storage instructions

The test kit must be stored at 2-8°C and can be used after opening until the expiry date printed on the label. As long as the diluted wash buffer is stored at 2-8 °C, it can be used for a maximum of 4 weeks. Microbial contamination must be prevented. After the expiry date has been reached, the quality guarantee is no longer valid.

It is imperative that the conjugate is prevented from contaminating the substrate solution because this will discolour the substrate. The substrate must also be protected from direct light in order to prevent decomposition or discolouration due to hydrolysis. Once discoloured, the substrate must not be used. See also Section 10. Quality control – indications of reagent expiry.

6. Materials required but not provided

6.1. Reagents

- Distilled or deionised water

6.2. Accessories

- Photometer for microwell plates (405 and 620 nm filters)
- Measurement range 0 to 3.5 OD.
- Washing machine for microwell plates or manual washing equipment (wash comb, pump, buffer stock and waste containers)
- 37°C incubator
- Micropipette 10-100 µl
- Micropipette variable up to 1000 µl
- Eppendorf tubes

- 8-channel or multi-step pipette
- Measuring cylinder (1000 ml)

7. Precautions for users

For *in vitro* diagnostic use only.

This test must only be carried out by trained laboratory personnel. The guidelines for working in medical laboratories must be followed and the instructions for carrying out the test must be strictly adhered to.

Samples or reagents must not be pipetted by mouth and contact with injured skin or mucous membranes must be prevented. When handling the samples, wear disposable gloves and when the test is finished, wash your hands.

Do not smoke, eat or drink in areas where samples or test reagents are being used.

The control sera (standard sera, high and low controls) in the kit have been tested for HIV- and HCV-Ak as well as HbsAg and syphilis CFR 21.640 with negative results. Nevertheless, they must be treated as potentially infectious in the same way as the patient samples and all other materials with which they come into contact and they must be handled in accordance with the relevant national safety regulations.

The wash buffer concentrate contains sodium azide as a preservative. Do not allow this substance to come into contact with the skin or mucous membranes. Explosive metal azides may be produced on contact with lead or copper pipes.

You are solely responsible for the proper disposal of all components in the kit after use.

All reagents and materials which come with potentially infectious samples must be treated with suitable disinfectants or heated in an autoclave at 121 °C for at least 1 hour.

8. Collection and storage of samples

The test has been developed for testing human serum. After blood collection, the blood should be separated from blood clots as soon as possible in order to prevent haemolysis. The samples must be stored cold or frozen until they are tested. Repeated freezing and thawing of the serum or microbial contamination must be avoided at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid sera can lead to false results.

Table 3: Sample storage

Undiluted serum		Diluted serum
2-8 °C	-20 °C	2-8 °C
1 week	>1 week	7 hours

9. Test procedure

9.1. General information

All reagents, patients' sera and the microwell plate must be brought to room temperature (20-25 °C) before use. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage at 2-8 °C.

The standard, control and allergen discs cannot be used more than once. The reagents and discs must not be used if the packaging is damaged or the containers are not sealed.

Components from kits with different lot numbers must not be combined or exchanged.

Using incubation times and temperatures other than those specified will cause the standard curve to be displaced from the standard curve on the certificate. Significant differences in the values on the standard curve will lead to invalid test results.

The test must not be carried out in direct sunlight. It is recommended that the microwell plate is covered.

The microwell plate must not be incubated in a damp chamber.

9.2. Preparing the wash buffer

Make up each bottle of AllergyWB wash concentrate to 1000 ml with distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37°C.

9.3. Filling the microwell plate with the allergen discs

The microwell plate Plate is supplied with the discs, DiscS, DiscC and DiscA , which are already in place according to the customer record.

When using allergen discs supplied in break-apart bars (A0049), the wells containing the allergen discs DiscA should be inserted in the frame supplied with the reagent kit after breaking apart the bars according to the operating record. The standard discs DiscS and control discs DiscC are already in the correct position on the frame in the reagent kit.

When performing panel tests (A0630), please note that panels of 16 allergens each are already inserted on the frames. However, different panels can be processed together on the same plate. In order to do this, simply replace the panels already on the plate with the desired panels.

Note: Only full plates should be processed because the reagent volumes are calculated accordingly.

9.4. Preparing the patient sera

The patient sera must be diluted with the sample dilution buffer Diluent 1:100. We recommend using at least 10 µl serum.

9.5. First incubation

Remove the sealing tape from the plate. When doing so, ensure that none of the allergen discs stick to and are removed with the tape.

The buffer which is present in the wells must be completely removed by suction. Pipette 50 µl of each of the standard sera Standard 1, Standard 2, Standard 3 and Standard 4, the high control Control high, the low control Control low and the diluted patient´s serum into the wells according to the pipetting scheme. After this, cover the plate Plate and incubate it at 37 °C for 60 minutes.

9.6. Washing

Wash the wells by filling them with diluted wash buffer and removing it by suction. Repeat this procedure six times. The last suction should take place as quantitatively as possible with the discs remaining in the wells.

When using a washing machine (which is recommended), 700 µl diluted wash buffer is used to wash the wells while removing it by suction at the same time (using a suitable overflow setting). It must be ensured beforehand that the unit is correctly adjusted to the dimensions of the type of plate being used.

When using a manual washing system, work with maximum volumes during each stage. Do not use pipettes for washing.

In order to improve washing efficiency, the jet of liquid should be aimed at the edge of the disc during both manual and automatic washing so that discs rotate on their own axis as the wash buffer is added.

Do not reduce the number of washing steps or the volume of washing buffer!

9.7. Second incubation

Pipette 50 µl conjugate Conjugate into each loaded well. After this, cover the plate Plate and incubate at 37°C for 60 minutes.

9.8. Washing

Washing – see Section 9.6.

9.9. Third incubation and measurement

Quickly pipette 100 µl substrate solution AllergySub S into each of the loaded wells. After this, cover the plate Plate and incubate it at 37 °C for 30 minutes. Stop the reaction by adding 50 µl stop reagent AllergyStop R to each well. Measure the extinction through the allergen discs at 405 nm against a reference wavelength of 620 nm. The plate can be measured again within 24 hours providing it has been covered and stored cold.

10. Quality control – indications of instability or deterioration

For each test carried out, test Standards 1-4 must be measured as well as a high and low control at the same time for the quality control (in duplicate). The test has been carried out correctly if the $OD_{405/620}$ for Standard 4 is greater than 1.0 and the IgG classes determined at 405/620 nm for the controls are within the range stated on the enclosed certificate.

If the values differ from those required or if the reagent is turbid or the substrate has turned yellow before adding to the wells, it may indicate that the reagents have expired.

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

- Expiry date of the reagents used
- Functionality of the equipment being used (e.g. calibration)
- Correct test procedure
- Visual inspection of the kit components for contamination or leaks – a substrate solution which has turned yellow must not be used.

If the conditions are still not fulfilled after repeating the test, please contact your local R-Biopharm distributor.

We recommend that you always carry out the test on sera for which you already have data at the same time and document the determined concentrations in µg/ml or IgG-classes in each case.

11. Evaluation and interpretation

11.1. Findings for the sera

11.1.1. Bases for the calculations

A reference curve must be set up in order to evaluate the test. This is why the standards are tested in duplicate on each plate simultaneously. In order to set up the reference curve, the average extinctions from the double determinations of the standards are plotted semi-logerithmically (x-log/y-lin presentation) as a function of the concentration (µg/ml) in a point-to-point presentation. The standard curve can be used to determine the concentrations of the specific IgG antibodies from the measured OD values. These can then be converted into IgG classes (see Table 4). The evaluation can also be carried out using the appropriate software.

The standard curve for RIDASCREEN® Spec. IgG is calibrated against the international reference preparation “1st WHO IRP 67/86 for human IgG”.

11.1.2. Concentrations, IgG classes and calculations

Table 4: Relationship between the determined µg/ml, IgG classes and antigen-specific IgG titres of the patient serum

µg / ml	IgG class	Allergen-specific IgG content
< 7,49	**0** (0.0 – 0.9)	negative
7.50 – 12,49	**1** (1.0 – 1.9)	low
12,5 – 19,99	**2** (2.0 – 2.9)	increased
20 – 49,99	**3** (3.0 – 3.9)	high
50 - 200	**4** (4.0 – 4.9)	very high

11.1.3 Interpretation of results for multi-allergen discs

Table 5: Relationship between the determined µg/ml and evaluation of the patient´s serum with multi-allergen discs

µg/ml	*Evaluation*
< 10	negative
≥ 10	positive

If the result is positive, the affected serum should be tested for specific reactions to the corresponding individual allergens.

12. Limitations of the method

The IgG concentrations determined by using this test system make it possible to say something about the degree of sensitisation of the patient to the individual allergens tested.

They cannot be used to derive a relationship between the determined IgG concentration and occurrence of serious clinical symptoms. The results obtained must always be interpreted in combination with the complete clinical picture.

With foods, in spite of the symptoms appearing during consumption, an increased IgG titre may not be found. The reason for this can be that IgG antibodies are directed towards epitopes which do not appear until the food is digested or may first appear during the industrial processing of the food and will therefore not be present on the allergen disc.

No IgG subclasses can be determined using this test.

False positive test results may be produced by cross reactivity of the antigens being tested with other antigens.

It cannot be ruled out that the epitopes which trigger the allergy are missing when the allergen discs are manufactured and this can also lead to false negative results.

13. Performance characteristics

Table 6: Intra-assay variation, all calculations are based on the ODs determined.

Intra-assay	**Lower range**	**Middle range**	**Upper range**
Allergens tested	A70, B7, E78, F1, F13, F33, F4,	E78, E11, E7, F183, I1, I3, M20	B7, F1, F4, F17, F2, M19, M2
No. Repl./allergen	24	24	24
Arithm. mean of VKs	7.68 %	6.72 %	5.22 %

Table 7: Inter-assay variation, all calculations are based on the ODs determined.

Inter-assay	**Lower range**	**Middle range**	**Upper range**
Allergens tested	A70, B7, E78, F1, F13, F33, F4, M19	B7, E11, E7, E78, F2, I1, I3, M20	F1, F17, F183, F4, M2
No. Repl./allergen	4 x 4	4 x 4	4 x 4
Arithm. mean of VKs	8.30 %	8.47 %	5.54 %

References

1. Harald Renz, Wolf-Meinhard Becker, Albrecht Bufe, Jörg Kleine-Tebbe, Monika Raulf-Heimsoth, Joachim Saloga, Thomas Werfel, Margitta Worm: In-vitro-Allergiediagnostik Positionspapier der Deutschen Gesellschaft für Allergologie und klinische Immunologie. Allergo Journal 2002; 11; 492-506.