R-Biopharm AG

# RIDA® AllergyScreen®

Allergen-Panels zur Bestimmung
des spezifischen IgE in Humanserum

*Allergy Panels for analysing*
*specific IgE in human serum*

## RIDA® AllergyScreen® – für die in vitro-Diagnostik

### Das Testprinzip: Der Immunoblot

Allergiespezifische Laboruntersuchungen sind ein unverzichtbarer Bestandteil der allergologischen Diagnostik. Zur Unterstützung Ihrer allergologischen Diagnostik im Laborbereich möchten wir Sie über das neuartige Screening-Konzept des RIDA® AllergyScreen® informieren.

Folgendes Prinzip liegt dem System zugrunde: auf die Oberfläche von Nitrocellulosemembranen sind speziell für die in-vitro-Diagnostik extrahierte Allergene gebunden. Dazu werden diese Allergene über ein Kontakt-Plotsystem auf die Nitrocellulose aufgetragen.

Im Gegensatz zu den meisten anderen Verfahren, bei denen einzelne Allergene kovalent an eine Matrix gekoppelt sind, werden bei diesem System Allergene als nativer, konzentrierter Extrakt passiv gebunden. Eine derartige Technik wird auch bei der Erkennung immunologischer Reaktionen in der Western-Blot-Analyse verwendet, so dass das Testverfahren auch als Immunoblot bezeichnet werden kann.

Auf einer Nitrocellulosemembran können beim RIDA® AllergyScreen® maximal 20 verschiedene Allergene aufgetragen werden.

Der Vorteil gegenüber Bestimmungen in Einzelsystemen liegt in der einfachen und schnellen Analyse einer ganzen Palette von Allergenen in einem Arbeitsgang. Es werden nur 250 µl Serum benötigt, die zur Erfassung eines spezifischen Sensibilisierungsmusters des Patienten ausreichen. Die Nitrocellulosemembranen befinden sich in einem Kunststofftrog, in dem nacheinander alle Arbeits- und Inkubationsschritte erfolgen. In diesen Reaktionstrog werden das Patientenserum und die Inkubationslösungen nacheinander pipettiert.

Mit Hilfe von 2 Haltekämmen, die je 10 dieser Tröge aufnehmen, lassen sich bis zu 20 Patientenseren bequem manuell in einem Arbeitsgang testen.

Notwendig zur Abarbeitung ist ein Kipp- oder Horizontalschüttler zur gleichmäßigen Verteilung der Lösungen auf den Membranen.

In dem zu untersuchenden Serum reagieren die allergenspezifischen IgE-Antikörper mit dem entsprechenden Allergen und werden spezifisch auf der Nitrocellulose gebunden. Nicht gebundene Antikörper werden durch Waschen entfernt.

Danach erfolgt die Zugabe eines mit Biotin gekoppelten anti-human-IgE Antikörpers, der an das jeweilige spezifische IgE aus der ersten Inkubation bindet. Nicht gebundener Detektorantikörper wird nach der Inkubation durch Waschen entfernt. Anschließend erfolgt die Zugabe eines mit alkalischer Phosphatase konjugierten Streptavidins. Dieses bindet an alle Biotinmoleküle des Detektors auf den Banden. Danach wird erneut gewaschen, um nicht gebundenes Streptavidinkonjugat zu entfernen.

Nach der Zugabe des Substrats (BCIP/NBT) erfolgt eine spezifische enzymatische Farbreaktion der alkalischen Phosphatase, wodurch positive Banden durch die Bildung von Farbstoffpräzipitaten auf der Nitrocellulosemembran sichtbar werden. Die Präzipitatbildung ist direkt proportional zum Gehalt des spezifischen IgE's in der Serumprobe des Patienten.

Die Auswertung erfolgt nach vollständiger Trocknung der Testmembran im RIDA® X-Screen, RIDA® quadro-Screen oder RIDA® maXi-Screen 2. Dabei handelt es sich um Geräte mit einer CCD-Kamera, die ein Photo der Membran aufnehmen.

Jedes Allergen wird aufgrund der Dichte der Bande, die integral vermessen wird, mithilfe der Standardkurve, die in der Software hinterlegt ist, befundet und in EAST-Klassen (0-6) eingestuft.

Diese EAST-Klassen stellen einen Bezug zum spez. IgE-Gehalt der Probe dar.

RIDA® X-Screen (single)

RIDA® quadro-Screen

RIDA® maXi-Screen 2


## *RIDA® AllergyScreen® – for in-vitro diagnostic*

### *Test principle: the immuno-blot analysis*

*The prevalence of allergies is increasing worldwide. In Europe alone up to 15 % of the population is thought to be afflicted.*

*Advances in allergy-specific laboratory tests are now indispensable tools for the diagnosis of allergies. It is necessary to test a patient's blood serum whenever an allergic response or severe reaction is suspected. Patients show often a wide range of varying symptoms and sensitization patterns against several allergens.*

*The RIDA® AllergyScreen® system is an in vitro system for proving the presence of specific IgE antibodies in serum. We would like to give you some information about this new screening concept: RIDA® AllergyScreen®.*

*Allergens, prepared only for in-vitro diagnostic purpose, are bound as test lines to the surface of nitrocellulose membranes which are glued after production in reaction troughs. The RIDA® AllergyScreen® system supports up to 20 different allergens on one membrane.*

*For the immunological reaction 250 µl of patient serum are pipetted into the trough and incubated at room temperature. During this time, the allergen-specific IgE-antibodies react with the allergen bands and bind to the nitrocellulose membranes via the allergen. Non-bound material is removed by washing.*

*After this, an anti-human IgE antibody coupled with biotin is added and incubated at room temperature. This binds to the bound specific IgE antibodies in the test fields from the first incubation. Non-bound detection antibodies are removed by washing.*

*Next, a streptavidin is added which is conjugated with alkaline phosphatase and incubated at room temperature. The streptavidin binds to the biotin from the second incubation in the test fields and to the positive control.*

*Non-bound streptavidin conjugate is removed by washing. After adding the substrate (BCIP/NBT) and incubating at room temperature, a specific enzymatic colour reaction of the alkaline phosphatase takes place. Positive bands become visible as a result of the formation of precipitates on the test membrane. The colouration is directly proportional to the specific antibody content of the serum sample.*

*Using 2 membrane panel holders, able to carry 10 panels each, 20 patient sera can be manually tested in one single test run.*

*For the performing of the test a shaker with reciprocal or rocking motion is necessary for an even distribution of the reagents over the test membrane.*

*Evaluation is carried out after complete drying of the test membrane with an instrument including a CCD camera (RIDA® X-Screen, RIDA® quadro-Screen or RIDA® maXi-Screen 2), which takes a photo of the membrane. The colour intensity of each allergen band is evaluated in accordance with the standard curve which is integrated in the software. The specific IgE concentrations are calculated in IU/ml and assigned to EAST classes (0-6).*





r-biopharm

## Zum Produkt

### Lieferbare Allergene

Es stehen unterschiedliche Panels mit speziellen Allergenzusammensetzungen von jeweils bis zu 20 Allergenen auf einer Membran zur Verfügung, die eine umfassende Bestimmung des spezifischen IgE's der Patienten erlauben.
Der Vorteil gegenüber Bestimmungen in Einzelsystemen liegt in der einfachen Analyse einer ganzen Palette von Allergenen in einem Arbeitsgang, wobei nur 250 µl Serum für 20 Allergene notwendig sind.
Die Testdauer beträgt 2,5 Stunden.

Das **Panel 1** ist eine Mischung aus 20 der wichtigsten Inhalations- und Nahrungsmittelallergenen zum effektiven Screening einer möglichen Sensibilisierung.

Das **Panel 2** (Inhalation) enthält 20 der wichtigsten inhalativen Allergene. Diese decken die wichtigsten inhalativen Sensibilisierungen ab.

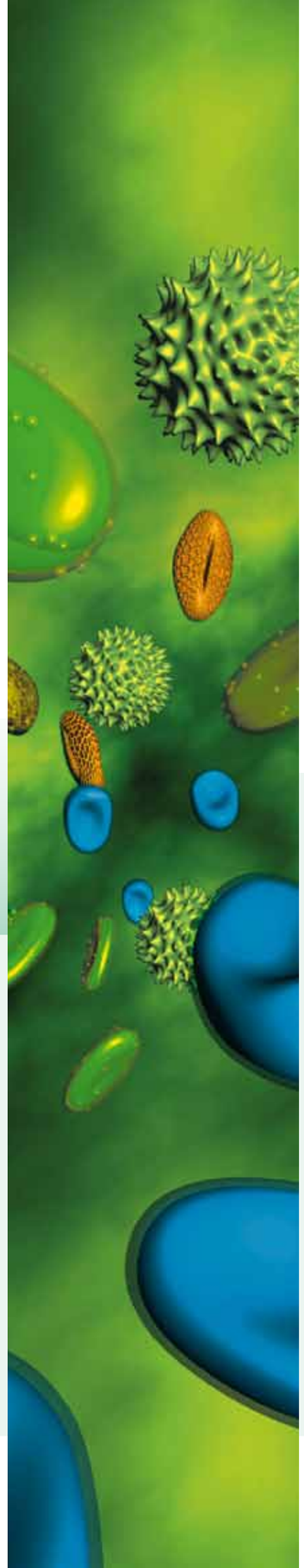
Das **Panel 3** umfasst eine Auswahl von 20 Nahrungsmittelallergenen, die als Hauptallergene bei der Diagnostik einer Nahrungsmittelallergie zu bewerten sind.

Das **Panel 4** deckt eine Auswahl von 20 inhalativen bzw. Nahrungsmittelallergenen mit besonderer Berücksichtigung von Kleinkindern und deren Nahrungsmittel ab.

Die Ergebnisse werden sowohl in IU/ml (von < 0.35 bis 100 IU/ml) als auch in Klassen (0-6) ausgegeben.
Damit ergibt sich eine rationelle und effiziente Allergieabklärung mit einer hohen Wirtschaftlichkeit im Vergleich zur Einzelbestimmung des spez. IgE's.

**Sie erhalten so in kürzester Zeit weitreichende Informationen über den Atopiegrad der Patienten und ein individuelles Sensibilisierungsmuster.**

RIDA® X-Screen (single)



## *The product*

### *Available allergens*

*There are different panels with specific compositions of up to 20 allergens available.*
*The advantage of RIDA® AllergyScreen® compared to a single allergen test system is the simple analysis of a whole range of allergens in one operation, whereby only 250 µl of serum are necessary for 20 allergens. The assay time is only 2.5 hours.*

***Panel 1** is a mixture of inhalative and food allergens for an efficient screening of a possible sensitization.*

***Panel 2** includes the most important inhalative allergens which covers the most important inhalative sensitizations.*

***Panel 3** contains only food allergens, which are characterized as most common allergens.*

***Panel 4** contains a selection of 20 inhalative and food allergens taking special consideration of children and their food.*

*The results are printed out in IU/ml (range < 0,35 - 100 IU/ml) as well as in EAST classes (0-6). In this way, an efficient and economic determination of specific IgE is given compared to a single allergen test system.*

***Please ask for specific allergen panels for your country !***



### Zusammensetzung der AllergyScreen Standard-Panels

| **Panel 1** Inhalative- und Nahrungsmittelallergene | **Panel 2** Inhalative Allergene | **Panel 3** Nahrungsmittel-Allergene | **Panel 4** Paediatrisches Panel |
|---|---|---|---|
| Pos. Kontrolle | Pos. Kontrolle | Pos. Kontrolle | Pos. Kontrolle |
| 1 Der. pteronyssinus | 1 Der. pteronyssinus | 1 Haselnuss | 1 Der. pteronyssinus |
| 2 Der. farinae | 2 Der. farinae | 2 Erdnuss | 2 Der. farinae |
| 3 Erlenpollen | 3 Erlenpollen | 3 Walnuss | 3 Birkenpollen |
| 4 Birkenpollen | 4 Birkenpollen | 4 Mandel | 4 Gräsermischung |
| 5 Haselpollen | 5 Haselpollen | 5 Milch | 5 Katze |
| 6 Gräsermischung | 6 Eichenpollen | 6 Eiweiß | 6 Hund |
| 7 Roggenpollen | 7 Gräsermischung | 7 Eigelb | 7 Alternaria alternata |
| 8 Beifuß | 8 Roggenpollen | 8 Kasein | 8 Milch |
| 9 Wegerich | 9 Beifuß | 9 Kartoffel | 9 α-Lactoalbumin |
| 10 Katze | 10 Wegerich | 10 Sellerie | 10 β-Lactoglobulin |
| 11 Pferd | 11 Katze | 11 Karotte | 11 Casein |
| 12 Hund | 12 Pferd | 12 Tomate | 12 Eiweiß |
| 13 Alternaria alternata | 13 Hund | 13 Kabeljau | 13 Eigelb |
| 14 Eiweiß | 14 Meerschweinchen | 14 Krabbe | 14 Rinderserumalbumin |
| 15 Milch | 15 Hamster | 15 Orange | 15 Sojabohne |
| 16 Erdnuss | 16 Kaninchen | 16 Apfel | 16 Karotte |
| 17 Haselnuss | 17 Pen. notatum | 17 Weizenmehl | 17 Kartoffel |
| 18 Karotte | 18 Cladosp. herbarum | 18 Roggenmehl | 18 Weizenmehl |
| 19 Weizenmehl | 19 Asp. fumigatus | 19 Sesam | 19 Haselnuss |
| 20 Sojabohne | 20 Alternaria alternata | 20 Sojabohne | 20 Erdnuss |
| Bestell-Nr. A2142 | Bestell-Nr. A2242 | Bestell-Nr. A2342 | Bestell-Nr. A2442 |

### *Composition of AllergyScreen standard panels*

| ***Panel 1*** *inhalative- und food allergens* | ***Panel 2*** *inhalative allergens* | ***Panel 3*** *food-allergens* | ***Panel 4*** *paediatric panel* |
|---|---|---|---|
| *Pos. control* | *Pos. control* | *Pos. control* | *Pos. control* |
| *1 Der. pteronyssinus* | *1 Der. pteronyssinus* | *1 Hazelnut* | *1 Der. pteronyssinus* |
| *2 Der. farinae* | *2 Der. farinae* | *2 Peanut* | *2 Der. farinae* |
| *3 Alder pollens* | *3 Alder pollens* | *3 Walnut* | *3 Birch pollens* |
| *4 Birch pollens* | *4 Birch pollens* | *4 Almond* | *4 Grass pollens* |
| *5 Hazel pollens* | *5 Hazel pollens* | *5 Milk* | *5 Cat* |
| *6 Grass pollens* | *6 Oak pollens* | *6 Egg white* | *6 Dog* |
| *7 Rye pollens* | *7 Grass pollens* | *7 Egg yolk* | *7 Alternaria alternata* |
| *8 Mugwort* | *8 Rye pollens* | *8 Casein* | *8 Milk* |
| *9 Plantain* | *9 Mugwort* | *9 Potatoe* | *9 α-Lactoalbumin* |
| *10 Cat* | *10 Plantain* | *10 Cellery* | *10 β-Lactoglobulin* |
| *11 Horse* | *11 Cat* | *11 Carrot* | *11 Casein* |
| *12 Dog* | *12 Horse* | *12 Tomato* | *12 Egg white* |
| *13 Alternaria alternata* | *13 Dog* | *13 Cod fish* | *13 Egg yolk* |
| *14 Egg white* | *14 Guinea pig* | *14 Crab* | *14 Bovine serum albumin* |
| *15 Milk* | *15 Golden hamster* | *15 Orange* | *15 Soya bean* |
| *16 Peanut* | *16 Rabbit* | *16 Apple* | *16 Carrot* |
| *17 Hazelnut* | *17 Pen. notatum* | *17 Wheat flour* | *17 Potatoe* |
| *18 Carrot* | *18 Cladosp. herbarum* | *18 Rye flour* | *18 Wheat flour* |
| *19 Wheat flour* | *19 Asp. fumigatus* | *19 Sesame seed* | *19 Hazelnut* |
| *20 Soya bean* | *20 Alternaria alternata* | *20 Soya bean* | *20 Peanut* |
| *Order-No. A2142* | *Order-No. A2242* | *Order-No. A2342* | *Order-No. A2442* |

## Auswertung

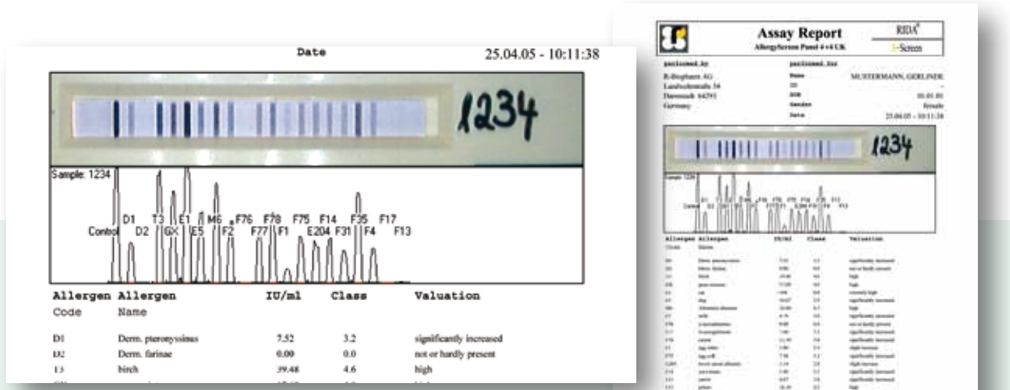
Die Auswertung erfolgt nach vollständiger Trocknung der Testmembran mittels RIDA® X-Screen, RIDA® quadro-Screen oder RIDA® maXi-Screen 2. Die eingesetzte Software ist von der digitalen Bildauswertung von Westernblotbanden abgeleitet. Dazu wird die Testmembran von der CCD-Kamera des Auswertesystems fotografiert. Ein Softwareprogramm wertet die Farbintensität der Allergenbanden in bestimmten Erwartungsfeldern, die für die Allergenbanden festgelegt sind, aus.

Dazu wird das berechnete Flächenintegral jeder Bande an einer in der Software hinterlegten Standardkurve abgeglichen.

Mit Hilfe eines mathematischen Algorithmus werden die Dichtewerte in IU/ml umgerechnet und in EAST-Klassen von 0-6 eingruppiert.

Mit dem Ausdruck nach der Messung erhält der Anwender ein Photo der Membran, die Densitometerkurve der Membran, die Konzentrationsangaben zu jeder Allergenbande in IU/ml sowie die entsprechenden Klassen.

Die Daten werden im System patientenspezifisch gespeichert, dokumentiert und sind jederzeit abrufbar. Alternativ kann die Auswertung mittels einer Farbkarte erfolgen.







## *Analysis*

*The basis of development of the software is the digital photographic evaluation of Western Blot bands. The RIDA® X-Screen, RIDA® quadro-Screen and RIDA® maXi-Screen 2 take photographs of the test membranes with a CCD camera and the software evaluates the colour intensity of the allergen bands using a mathematical algorithm.*

*This is achieved by comparing the calculated surface integral of each band with an internal standard curve and grouping these results into classes (0-6). Classes are directly related to the specific IgE content in IU/ml.*

*After measurement a printout provides the user with a photo of the membrane, the densitometer curve of the membrane, the concentration data for each allergen band in IU/ml and the according classes. The data are stored and documented in the system, specifically for each patient, and are retrievable. Additional the analysis can be done visually by the use of a colour card.*



## Automatisierung

Die Testdurchführung kann durch den Bee Blot der Firma Bee Robotics ergänzt und so 36 RIDA® AllergyScreen® Membranen automatisiert entwickelt werden. Die Kombination aus Bee Blot und RIDA® maXi-Screen 2 erfüllt alle Ansprüche an ein automatisiertes System, wie Handlingsicherheit, keine Verwechslungsgefahren durch manuellen Transfer der Membranen, online-Datenübertragung vom LIS-System zum Reader und Rückübertragung der Ergebnis in das LIS-System. Dadurch wird das System auch für Laboratorien mit größerem Probendurchsatz einsetzbar.

Nur 250 µl Serum müssen in die Reaktionströge manuell eingefüllt werden. Nach Prozessende und Trocknung wird der gesamte Membranhalter mit den fertig entwickelten Membranen in den RIDA® maXi-Screen 2 eingesetzt und ausgewertet.

Der RIDA® quadro-Screen ist ebenfalls für die online-Datenübertragung ausgelegt.

RIDA® maXi-Screen 2

Bee Blot

## *Automation*

*With the help of an immunoblot incubator like the Bee Blot from Bee Robotics 36 RIDA® AllergyScreen® panels can be developed automatically. The combination of Bee Blot with RIDA® maXi-Screen 2 avoids handling failures and the mix-up of panels due to manual transfers. RIDA® maXi-Screen 2 and RIDA® quadro-Screen support online connection to LIS systems offering work list management and transfer of results back to the LIS.*

*Therefore the RIDA® AllergyScreen® system will become useful for high throughput laboratories leading to enhanced efficiency and ease of performance of allergy testing.*

*Only 250 µl patients sera have to be pipetted manually into the reaction troughs. After the incubation steps and drying the whole comb with 36 RIDA® AllergyScreen® panels can be transferred into the RIDA® maXi-Screen 2 for automated evaluation.*

**R-Biopharm AG wurde 1988 als Tochter der Röhm AG in Darmstadt gegründet. 1991 wurde R-Biopharm von Herrn Dr. Ralf Dreher durch ein „Management-Buy-Out" erworben. Die Hauptaktivitäten liegen in der Entwicklung, Produktion und Vermarktung von 2 Produktlinien.**

**1. Lebens- und Futtermittelanalytik**
Diese Produktlinie besteht aus Testsystemen zum schnellen Nachweis von Mykotoxinen, Hormonen und Anabolika, Antibiotika, Vitaminen, Nahrungsallergenen und einigen anderen Parametern.

**2. Enzym Immuno Assays zum Nachweis von humanen Infektionskrankheiten**
Diese Produktlinie beinhaltet Testsysteme im Bereich Stuhldiagnostik, Infektionskrankheiten, serologische und allergologische Diagnostik.

Alle Produkte sowohl für die Lebens- und Futtermittelanalytik als auch für die klinische Diagnostik werden weltweit unter den Handelsnamen RIDASCREEN® und RIDA® über ein internationales Distributorennetz oder durch eigene Tochterunternehmen wie in USA (R-Biopharm Inc.), Grossbritannien (R-Biopharm Rhône Ltd), Frankreich (R-Biopharm France), Italien (R-Biopharm Italia), Argentinien (R-Biopharm Latinoamérica), Brasilien (R-Biopharm Brasil), Spanien (R-Biopharm España), China (R-Biopharm Analysis Systems Trading Co. Ltd.) und Australien (R-Biopharm Australia) vertrieben.

Seit November 1996 ist das Qualitätsmanagement von R-Biopharm nach DIN EN ISO 9001 und DIN EN 46001 (Medizinprodukte) zertifiziert. 2003 erfolgte die Zertifizierung nach der Qualitätsmanagement-Norm ISO 13485 (Medizinprodukte). Seit Januar 2000 ist R-Biopharm der weltweit exklusive Distributor für die enzymatischen Tests von Roche (ehemals Boehringer Mannheim).

***R-Biopharm AG was founded in 1988 as a subsidiary of the company Röhm AG in Darmstadt. In 1991 R-Biopharm was acquired by Dr. Ralf Dreher through a management buy out. The main activity is the development, production and marketing of two different product lines.***

***1. Enzyme immunoassays for the detection of residues in food and feed***
*This product line contains test systems for the rapid screening for mycotoxins, hormones and anabolics, antibiotics, vitamins, food allergens and some other parameters.*

***2. Enzyme immunoassays for the detection of human infectious diseases***
*The product line contains test systems in the field of stool diagnostics, infectious disease, serology and allergy testing.*

*All products, agri/food diagnostics as well as the clinical diagnostics, are distributed world-wide under the trademarks RIDASCREEN® and RIDA® via an international distributor network or through own daughter companies like in the US (R-Biopharm Inc.), in the UK (R-Biopharm Rhône Ltd), in France (R-Biopharm France), in Italy (R-Biopharm Italia), in Argentina (R-Biopharm Latinoamérica), in Brazil (R-Biopharm Brasil), in Spain (R-Biopharm España), in China (R-Biopharm Analysis Systems Trading Co. Ltd.) and in Australia (R-Biopharm Australia).*

*Since November 1996 the quality management of R-Biopharm is certifi ed according to DIN EN ISO 9001 and DIN EN 46001 (medical products). Certification according to the Quality Management Standard ISO 13485 (Medical product) took place in 2003. Since January 1st 2000 R-Biopharm is the world-wide exclusive distributor for the Boehringer Mannheim Roche enzymatic tests.*

Your local distributor:

R-Biopharm AG
An der neuen Bergstraße 17, 64297 Darmstadt, Germany
Phone: + 49 (0) 61 51 - 81 02-0
Fax: + 49 (0) 61 51 - 81 02-20 / -40
www.r-biopharm.com, E-mail: info@r-biopharm.de

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# RIDA® AllergyScreen

Article no: A2142 Panel 1 / ASAN / HVEN / IND /
IR / KO / KZ / MA / NP / OUSA / PE /TW / UY / VE / VIET /

Article no: A2242 Panel 2 / ASAN / CA / DOHA / H / IND /
KO / KSA / KZ / MA / ME / MENA / MOFID / TR / VE /

Article no: A2342 Panel 3 / CA / DOHA / H / IND / KO / KSA / KZ /
MA / ME / MENA / MOFID / RO / SK / TR / VE /

Article no: A2442 Panel 4 / KO / KZ / MA / VE

Article no: A3054

R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt,
Germany, Tel: +49 (0) 61 51 81 02-0 / Fax: +49 (0) 61 51 81 02-20

CE

## 1. Intended use

For *in vitro* diagnostic use. This is an enzyme immunoassay on a nitrocellulose membrane (immunoblot) for the semi-quantitative determination of specific IgE antibodies against a panel of individual allergens in human serum.

## 2. Summary and explanation of the test

The purpose of the immune system is to defend the body against pathogenic bacteria, viruses and other microorganisms. The defence reaction serves to protect the organism on initial contact with the pathogens and immunise it on repeated contact. All allergic reactions are preceded by an initial contact phase without symptoms during which Class E specific antibodies (IgE antibodies) are formed. On repeated contact with the allergens which trigger the reaction, these IgE antibodies react with the allergens and lead to the release of mediators (from mast cells or mastocytes) such as histamine, leucotrien and prostaglandine etc. which lead to the symptoms of the allergy. When there is an allergic reaction, the allergens causing the reaction can be identified by determining the specific IgE antibodies in the serum. This method can also be used to determine sensitisations without symptoms.

## 3. Test principle

This test is based on the principle of an enzyme immunoassay on a nitrocellulose membrane (immunoblot). The allergens which correspond to the panel composition are applied to the surface of nitrocellulose membranes. Allergen-specific IgE antibodies which are present in patient samples react with the antigens and attach themselves to biotin-coupled anti-human IgE antibodies during a second step. During the third incubation step, the Biotin attaches itself to a streptavidin conjugated with alkaline phosphatase (Conjugate). The enzyme converts the colourless substrate (BCIP/ NBT) to a blue/lilac end product in the last incubation step. Between the single incubation steps a washing step must be performed. The intensity of the blue colour is proportional to the quantity of allergen-specific antibodies in the serum. The evaluation is carried out by using an analysis template or with the RIDA® X-Screen / RIDA® quadro-Screen or RIDA® maXi-Screen 2.

## 4. Reagents provided

Table 1: Kits for manual execution:

| | | |
|---|---|---|
| Membrane | 10 pieces | RIDA® AllergyScreen test membrane (nitrocellulose membrane) coated with different allergens on 20 test fields in a reaction trough |
| Wash | 20 ml | Wash buffer, x 25 concentrate, Tris / NaCl |
| Antibody | 4 ml | Detector antibody; anti-human IgE antibodies (goat) conjugated with biotin, ready for use |
| Conjugate | 4 ml | Streptavidin conjugate; streptavidin conjugated with alkaline phosphatase, ready for use |
| Substrate | 4 ml | Substrate; BCIP/NBT (bromochloroindolyl phosphate / Nitro Blue Tetrazolium), ready for use |

Table 2: RIDA® AllergyScreen Reagent Kit (A3054)

| | | |
|---|---|---|
| Wash | 2 x 20 ml | Wash buffer, x 25 concentrate, Tris / NaCl |
| Antibody | 2 x 4 ml | Detector antibody; anti-human IgE antibodies conjugated with biotin, ready for use |
| Conjugate | 2 x 4 ml | Streptavidin conjugate; streptavidin conjugated with alkaline phosphatase, ready for use |
| Substrate | 2 x 4 ml | Substrate; BCIP / NBT (bromochloroindolyl phosphate / Nitro Blue Tetrazolium), ready for use |

## 5. Storage instructions

The test membranes must be stored in the plastic packaging under cool, dry, dark conditions. The test kit must be stored at 2-8 ℃ a nd 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 ℃, it can be used for a maximum of 4 weeks. Mi crobial 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 auto-oxidation. Once the colour has changed, the substrate must not be used.

## 6. Additional necessary reagents – and necessary equipment

6.1. Reagents

- Distilled or deionised water

6.2. Accessories

- Vortex mixer
- Measuring cylinder (500 ml)
- Micropipette 250 µl
- 500 ml Laboratory wash bottle
- Test membrane holder for holding 10 test-membranes (optional)
- Incubation box for incubation in the dark (system consisting of test membrane holder and incubation box can be obtained from R-Biopharm)
- Horizontal shaker (optional)
- Hairdryer, standard (optional)
- RIDA® X-Screen measuring instrument including software plus personal computer with USB port (optional)
- RIDA® quadro-Screen measuring instrument including computer and software (optional)
- RIDA® maXi-Screen 2 measuring instrument including computer and software (optional)

## 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.

Antibody and wash buffer concentrate contain sodium azide as a preservative. This substance must not be allowed to come into contact with the skin or mucous mem-

brane. Explosive metal azides may be produced on contact with lead or copper pipes.

Conjugate contains methylisothiazolone and bromonitrodexan in subtoxic concentrations as a preservative.

If the outer packaging is defective, the individual components must be examined to make sure they are undamaged before use. Kit components must not be used if their individual packaging is damaged or their containers are not sealed.

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

All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or autoclaved at 121℃ for at least 1 hour.

## 8. Specimen collection and storage

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 and microbial contamination must be avoided at all costs. Using heat-inactivated, lipaemic, haemolytic, icteric or turbid sera can lead to false results.

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Table 3: Sample storage

| Undiluted serum | |
|---|---|
| 2-8 ℃ | -20 ℃ |
| 1 week | >1 week |

## 9. Test procedure

9.1. General information

All reagents, patients' sera and test membranes must be brought to room temperature before use. The reagents must be thoroughly mixed immediately before use. After use, the kit must be immediately returned to storage at 2-8 ℃.

The test membranes cannot be used more than once. The reagents and test membranes 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.**

Reproducible results strongly depend on keeping to the incubation times and temperatures as well as washing the test membranes uniformly.

The test must not be carried out in direct sunlight. The test membranes must only be held by the handle. Contact with the reaction surface must be avoided – do not touch it. The handle of the reaction trough can be marked with patient data (e.g. laboratory numbers) – with water resistant felt-tip pen.

9.2. Preparing the wash buffer

Make up each bottle of wash buffer concentrate Wash to 500 ml with distilled water. Any crystals present in the concentrate must be dissolved beforehand by warming in a water bath at 37℃. Fill a laboratory wash bottle with diluted wash buffer.

9.3. First incubation

Take the test membranes Membrane out of the packaging according to the number of tests which are to be carried out. For simplicity, the test membrane holder which can hold 10 test membranes Membrane can be used. Wet the test membranes completely with diluted wash buffer (squeezing bottle) and wait until no tiny bubbles are rising up. This is most easily achieved by keeping the test membrane holder horizontal and, after filling the test membranes, carefully rocking the holder back and forth several times. After this, empty the test membranes Membrane, rinse them again briefly with diluted wash buffer and hit it upside down onto an absorbent tissue. Subsequently fill the test membranes Membrane with 250 µl patient serum and incubate them at room temperature (20-25 ℃) on the horizont al shaker (100 - 120 rpm) for 45 minutes. Please make sure that the membranes are covered completely by the fluid.

9.4. Washing

Rinse off the test membranes Membrane over the sink with the diluted wash buffer (squeezing bottle) for at least 5 seconds. Hold the test membranes Membrane vertically downwards to avoid splashing the sera onto neighbouring test membranes. Direct the jet of wash solution over the test membranes several times. After this, fill the Membrane with diluted wash buffer, shake it back and forth several times and empty. Finally, hold the test membranes Membrane downwards at an angle again and rinse them with the wash bottle for 5 seconds. After this, empty the test membranes and hit them upside down onto an absorbent tissue.

9.5. Second incubation

Add 5 drops (approx. 250 µl) antibody Antibody to each test membrane. Please make sure that the membranes are covered completely by the fluid. Incubate the test

membranes Membrane on the horizontal shaker (100 - 120 rpm) at room temperature (20-25 ℃) for 45 minutes.

9.6. Washing

Washing – see Section 9.4.

9.7. Third incubation

Add 5 drops (approx. 250 µl) conjugate Conjugate to each test membrane. Please make sure that the membranes are covered completely by the fluid. Incubate the test membranes Membrane on the horizontal shaker (100 - 120 rpm) at room temperature for 20 minutes.

9.8. Washing

Washing – see Section 9.4.

9.9. Fourth incubation

Add 5 drops (approx. 250 µl) substrate Substrate to each test membrane Please make sure that the membranes are covered completely by the fluid. Incubate the test membranes Membrane on the horizontal shaker (100 - 120 rpm) at room temperature in the dark for 20 minutes.

After incubation, terminate the colour reaction by briefly rinsing the test membrane membrane with plenty of distilled water or under running water (tap water). Leave the membranes to dry in the air or use a standard hairdryer to accelerate the drying process. The blue/lilac-coloured background on the test membranes disappears on drying. The test membranes in the reaction trough Membrane must not be evaluated until they are completely dry.

## 10. Quality control – indications of reagent expiry

The test has been correctly carried out if the background has fully disappeared and the positive control shows either a strong band or achieves at least EAST Class 2,5 when evaluated in the RIDA@ X-Screen, -quadro-Screen or -maxi-Screen 2.

If the values differ from those required or if the reagent is turbid or the substrate has turned blue before adding it to the test membranes, this may be an indication 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 blue must not be used.

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

## 11. Evaluation and interpretation

11.1. Membrane configurations of RIDA® AllergyScreen Panels 1, 2, 3, and 4

| Panel 1 | Panel 2 | Panel 3 | Panel 4 |
|---|---|---|---|
| Positive control | Positive control | Positive control | Positive control |
| Derm. pteronyssinus | Derm. pteronyssinus | Hazelnut | Derm. pteronyssinus |
| Derm. farinae | Derm. farinae | Peanut | Derm. farinae |
| Alder | Alder | Walnut | Birch |
| Birch | Birch | Almond | Grass mixture |
| Hazel | Hazel | Milk | Cat |
| Grass mixture | Oak | Egg white | Dog |
| Rye (Pollen) | Grass mixture | Egg yolk | Alternaria alternata |
| Mugwort | Rye (Pollen) | Casein | Milk |
| Plantain | Mugwort | Potato | α-Lactalbumin |
| Cat | Plantain | Celery | ß-Lactoglobulin |
| Horse | Cat | Carrot | Casein |
| Dog | Horse | Tomato | Egg white |
| Alternaria alternata | Dog | Cod | Egg yolk |
| Protein | Guinea pig | Crab | Bovine serum albumin |
| Milk | Hamster | Orange | Soybean |
| Peanut | Rabbit | Apple | Carrot |
| Hazelnut | Penicillium notatum | Wheat flour | Potato |
| Carrot | Cladospor. herbarum | Rye flour | Wheat flour |
| Wheat flour | Aspergillus fumigatus | Sesame | Hazelnut |
| Soybean | Alternaria alternata | Soybean | Peanut |

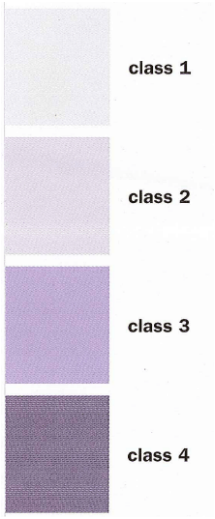
The membrane configurations for all other country specific panels to which this package leaflet applies are available with R-Biopharm AG as supplement for each panel.

## 11.2. Findings for the sera

### 11.2.1. Optical-visual assessment

The colour intensity on the test fields is directly proportional to the quantity of specific IgE antibodies in the serum of the patient for the allergen concerned.

If a band appears in relation to the membrane background, then specific antibodies are present in the serum. If no colouring appears in the reaction field on the membrane, then RIDA® AllergyScreen has not detected any IgE antibodies which are specific to this allergen.




The semi-quantitative evaluation can be carried out using the colour scale (Figure 2) over 4 classes. The grey scale (left side of the analysis template provided with each kit) should be used for direct comparison.

Figure 2: Grey scale for the semi-quantitative evaluation (classes 0 - 4)

Table 4: Relationship between the class determined and the allergen-specific IgE content of the patient serum

| Class | Allergen-specific IgE content |
|---|---|
| 0 | none found or hardly exists |
| 1 | low |
| 2 | increased |
| 3 | significantly increased |
| 4 | High |

11.2.2. Quantification using RIDA® X-Screen / RIDA® quadro-Screen / RIDA® maXi-Screen 2

For the quantification, the test membranes are inserted into the membrane holder of one of the above mentioned measuring instruments and measured using the associated software. The IU/ml are calculated automatically from the measured values and assigned to the test classes 0 - 6. The evaluation is based on a standard curve which is stored in the evaluation software.

**Please pay attention to the reader specific instructions when using one of the measuring instruments.**

**It is essential to make sure that the test which corresponds to the allergy panel concerned is called before the measurement.**

Using Table 5, the allergen-specific IgE titers can be read off from the concentrations determined in IU/ml or classes.

Table 5: Relationship between the determined IU/ml, classes and allergen-specific IgE contents of the patient serum

| IU/ml | EAST-Class | Allergen-specific IgE content |
|---|---|---|
| 0.00 - 0.34 | 0 (0.0 - 0.9) | none found or hardly exists |
| 0.35 - 0.69 | 1 (1.0 - 1.9) | low |
| 0.70 - 3.49 | 2 (2.0 - 2.9) | increased |
| 3.50 - 17.49 | 3 (3.0 - 3.9) | significantly increased |
| 17.50 - 49.99 | 4 (4.0 - 4.9) | high |
| 50.00 - 99.99 | 5 (5.0 - 5.9) | very high |
| ≥ 100.00 | 6 (≥ 6.0) | extremely high |

11.3. Documentation

After drying of the test membranes and evaluation in the RIDA® X-Screen / RIDA® quadro-Screen or RIDA® maXi-Screen 2 or by optical-visual means has been carried out, the membranes can be removed from the reaction troughs using tweezers and documented in an operating record.

The measuring data (photo of the test membranes and evaluation) are stored on the hard disk in the PC in a default directory. A data sheet can be printed out from a standard printer connected to the PC / measuring instrument for each serum tested.

## 12. Limitations of the method

The IgE 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 or mixtures of allergens tested.

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

Because of the absence of national and international standards and because of the possible differences in prick-test solutions and allergen extracts which are used for the *in-vitro* tests, it is possible for discrepancies to occur between the results from the *in-vivo* tests and results from the *in-vitro* tests. IgE titres measured immediately after the appearance of anaphylactic reactions may also be negative or too low. If there are discrepancies between the results from the *in-vivo* and *in-vitro* diagnostics, the test should be repeated after 3-4 weeks. Discrepancies which arise should be investigated by an allergologist with a follow-up *in-vivo* test such as a provocation test. Provocation tests can trigger anaphylactic shock.

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

## 13. Performance characteristics

Intra-assay variation: average 4,5 %

Inter-assay variation: average 4,8 %

In order to determine the sensitivity and specificity, 142 sera were tested (in 881 determinations altogether) as part of a clinical study in a quantitative reference *in-vitro* system and compared with the findings of the RIDA® Allergy-Screen. 737 skin-prick test results were also compared with the results of the RIDA® AllergyScreen-Tests.

Comparison with the IgE reference system:

Sensitivity: 84.3 %

Specificity: 95.0 %

Accuracy 90.6 %

Comparison with the skin prick test:

Sensitivity: 95.1 %

Specificity: 80.2 %

Accuracy 88.3 %

Literature


- Kersten, W., von Wahl, P.G., Lange, C.E., Wenning, J.: Empfehlungen zur in-vitro Diagnostik allergischer Erkrankungen, [Recommendations for the in-vitro diagnosis of allergic disorders] Allergologie, Jg. 23, No. 6, 304-307 (2000)
- von Wahl, P.G., Kersten, W.: Klinische Studie mit einem neuen in-vitro Testsystem, [Clinical study with a new in-vitro test system] Allergo Journal 8, 107 – 111 (1999)
- Kjellman, N.I.M.: Prediction and prevention of atopic allergy, Allergy 37, 463 - 473 (1982)
- Debelic, M.: Die klinische Bedeutung der Bestimmung von Serum-Gesamt IgE und spezifischem IgE für die Diagnostik und Verlaufsbeobachtung allergischer Krankheitsbilder [The clinical importance of determining total serum IgE and specific IgE in the diagnosis and monitoring of allergenic clinical pictures]. Therapiewoche 29, 2280 - 2295 (1979)
- Urbanek, R.: Papier-Radio-Immuno-Sorbent-Test (PRIST) - IgE-Spiegel bei nicht allergischen und allergischen Kindern, [Paper disc Radio Immuno Sorbent Test (PRIST) - IgE levels for non-allergic and allergic children] Monatsschrift Kinderheilkunde 125, 583 - 585 (1977)
- Kjellman, N.I.M., et al.: Serum IgE levels in healthy children quantified by a Sandwich technique (PRIST), Clinical Allergy 6, 51 - 59 (1976)
- Turner, K.J.: IgE globulins and immunity. Medical Journal of Australia 2, 846 (1974)
- Johannson, S.G.O.: Raised levels of a new immunoglobulin class (IgND) in asthma. Lancet II, 951 - 953 (1967)
- Ishizaka, K., Ishizaka, T., Hornbook, M.M.: Physico-chemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. Journqal of Immunol 97, 75 (1966)
- Bennich, H.H., et al.: Immunoglobulin E, a new class of human immunoglobulin. Bulletin World Health Organisation 38, 151 (1964)