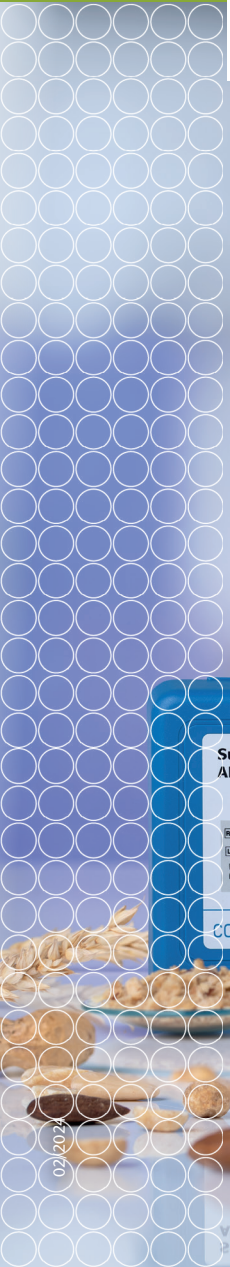


Food allergen testing solutions

from R-Biopharm



Qualitative and quantitative detection



Lateral Flow, ELISA, real-time PCR – manually & automated



HACCP, hygiene monitoring, product control

More information:



<https://r-b.io/allergens>

Allergen testing

What are food allergies?

A food allergy is a malfunction of the immune system against food ingredients. In most cases, the allergens are proteins. Generally, 2 - 4 % of adults and 6 - 8 % of children develop a food allergy. Allergenic reactions vary from mild to life threatening, depending on the allergen, its intake and the patient's situation. There is no cure for patients with an allergy. Even small traces of the

allergen can cause a severe reaction. Thus, the only way to keep patients safe is a strict avoidance of the foods in question.

Although celiac disease is often associated with a food allergy, it is a cell-mediated disease, where T-cell immune mechanisms lead to inflammation of the small intestine.

Why is labelling food so important?

Food allergen labeling should provide clear, accurate and complete information about ingredients. To protect sensitive consumers, allergenic food ingredients need to be declared on food labels. However, allergen labeling is not harmonized

internationally. As shown in the table, allergen labeling differs across countries. Analytical methods such as ELISA, lateral flow and real-time PCR are used to support correct labeling.





What are the present regulations for allergens in food?

There are currently no internationally defined thresholds for allergens. However, varying action levels for food authorities exist on a national basis e.g. in European countries, North America or Australia. To harmonize this situation, the WHO/FAO are in preparation of international

recommendations.

An exception is gluten, where defined thresholds exist. According to the Codex Alimentarius (CODEX STAN 118/1979), food products, which contain less than 20 mg/kg can be labeled as "gluten-free".

Table 1: International food allergen labeling regulations for selected countries.

Food allergens	USA	Canada	EU	Australia/NZ	Argentina	Malaysia	Japan
Crustacean shellfish	●	●	●	●	●	●	●
Egg	●	●	●	●	●	●	●
Fish	●	●	●	●	●	●	
Milk	●	●	●	●	●	●	●
Peanut	●	●	●	●	●	●	●
Soy	●	●	●	●	●	●	
Tree nut	●	●	●	●	●	●	●
Wheat	●	●	●	●			●
Cereals with Gluten		●	●	●	●	●	
Sulfites	●	●	●	●	●	●	
Buckwheat							●
Celery			●				
Lupin			●	●			
Molluscan shellfish		●	●	●			
Mustard		●	●				
Sesame	●	●	●	●			
Bee pollen/Propolis				●			
Royal jelly				●			

Lacteal secretion from cows
 ≥10 mg/kg
 Directly added or ≥ 10 mg/kg
 All farmed birds
 Milking animals
 From mammary gland of farmed animals

Source: <https://farrp.unl.edu/IRChart>

Allergen testing

At which stages are food allergens tested?

To ensure allergen free foods, a robust HACCP (Hazard Analysis and Critical Control Points) concept must be implemented at all stages of a food supply chain - "from farm to fork". Cross contamination of food can occur already on the fields or during transportation.

In food processing companies, a risk-based allergen management should include testing of raw materials, manufacturing areas as well as final products (see figure 1). Surfaces in the processing equipment

need to be cleaned, because line sharing is a risk for cross-contamination. Therefore, testing swabs from equipment after sanitation and/or rinse water is necessary to verify the efficacy of a cleaning procedure.

To support analysis at critical control points, R-Biopharm offers perfectly fitted test solutions like LFD, ELISA and real-time PCR providing both, qualitative and quantitative results as you need it.

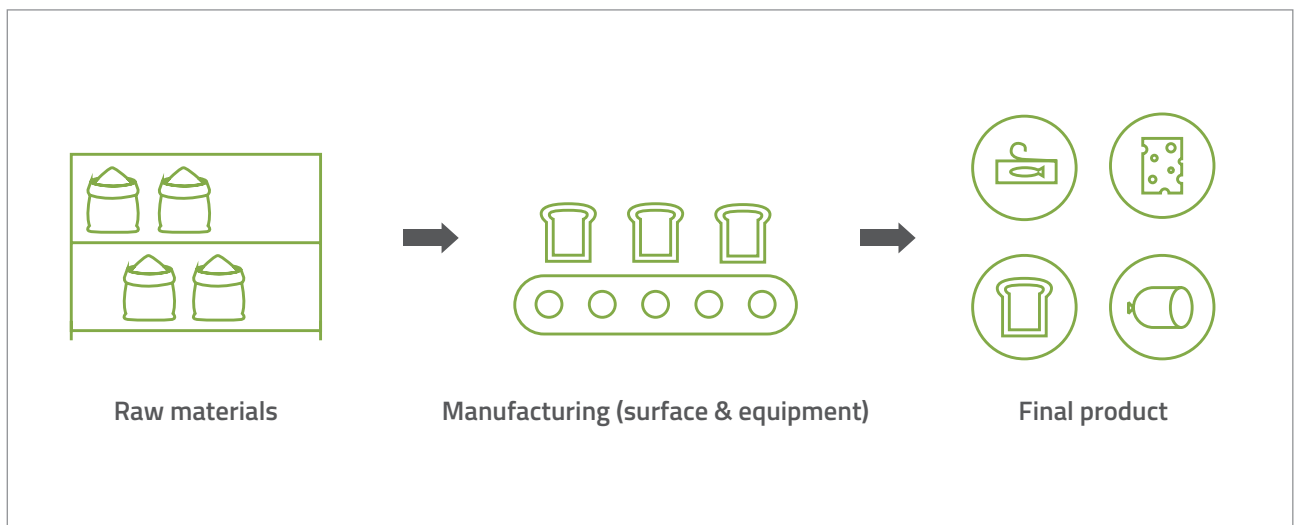


Figure 1: Testing for food allergens across all stages of the food supply chain – from farm to fork.

Allergen management
Infographic:



<https://r-b.io/22>

What do the different analytical methods detect?

Analytical methods such as the lateral flow device (LFD), enzyme-linked immunosorbent assay (ELISA) and real-time PCR (qPCR) are used for allergen testing. Real-time PCR detects DNA, while LFD and ELISA are immunobased methods, using antibodies to detect a protein component of the food. The

principle of a sandwich ELISA and LFD are the same: specific antibodies binding the target analyte(s) (here: allergenic protein(s)) are used. For measuring or visualization, the antibodies are labeled e.g. with an enzyme (ELISA) or gold/latex (LFD).



ELISA is a more laboratory-based technique where the antibodies are immobilized on a microtiter plate. After adding the sample and afterwards a substrate, a reaction occurs (color change). The intensity of the color varies depending on the amount of analyte present in the sample. This can be measured with a microplate reader and utilizing standard solutions, a quantification is possible. ELISA provides high sensitivity and accuracy but is more suitable for use in laboratories.



LFDs are a rapid and cost-effective method often used for point-of-care diagnostic, where no lab equipment is available. Here, the antibodies are immobilized on a membrane (strip), and the sample is passed through the strip. If the target analyte is present, a visible test line appears on the strip. This method does not require special equipment, is easy to perform, and provides quick results.



Real-time PCR is a more laboratory-based technique where DNA sequences of the allergenic species are detected. It allows highly specific and sensitive detection as well as quantification of the targeted allergenic food. It is a standardized procedure and allows running several tests simultaneously from one extracted sample.

Allergen testing

Which analytical methods are offered by R-Biopharm AG?

R-Biopharm offers different analytical methods: ELISA, LFD and qPCR. While ELISA and LFD detect proteins, qPCR detects the DNA. These methods are complementary. Each method shows specific

advantages within different fields of application. However, they can be used for reciprocal confirmation too.

RIDA®QUICK/bioavid

Lateral flow tests – Point of care

- On-site testing
- Simple & reliable
- Hygiene testing: swab test, CIP water
- Raw material and product control (foods and ingredients)
- No lab equipment required
- Rapid decision
- Simple documentation with RIDA®SMART App Allergen



RIDASCREEN®

ELISA – Laboratory method

- Quantitative results using recognized reference materials, if available (e.g. NIST)
- Simple sample preparation and test procedure
- Widely approved by independent organizations as AOAC, AACC, ICC
- Automation solutions available
- Evaluation with the user-friendly software – RIDASOFT® Win.NET



SureFood®ALLERGEN

Real-time PCR – Laboratory method

- Highly specific & sensitive
- Uniform sample preparation, handling and test procedure
- SureFood® PREP Advanced for manual or SureFast® Mag PREP for semi-automated DNA extraction
- Multiplex testkits available
- Quantify with SureFood® QUANTARD Allergen 40





Why is external validation by an independent body important?

The greatest benefit of external method validation is that it builds confidence for the end user and confirms highest quality. The validation exercise is costly and time consuming, but the generated data can be trusted. For example, AOAC International is a globally

recognized, independent association. The AOAC has two programs by which methods are evaluated and approved: AOAC Official Method of Analysis (OMA) and AOAC-RI Performance Tested Method (PTM).

R-Biopharm offers broadest range of independently approved methods:

RIDASCREEN® Gliadin

Art. No. R7001

- Codex Alimentarius Method (Type I)
- AOAC-OMA 2012.01 "Final Action" in food
- AOAC-RI 120601
- AACCI 38-50.01
- ICC 182

RIDASCREEN® Total Gluten

Art. No. R7041

- AOAC OMA 2018.15 "First Action"

RIDASCREEN®FAST Milk

Art. No. R4652

- AOAC-RI 101501

RIDASCREEN® Gliadin competitive

Art. No. R7021

- AOAC-OMA 2015.05 "Final Action"
- AACCI 38-55-01
- ICC 183

RIDA®QUICK Gliadin

Art. No. R7003/04/05

- AOAC-OMA 2015.16 "Final Action"
- AACCI 38-60.01
- AOAC-RI 101702

RIDASCREEN®FAST Peanut

Art. No. R4652

- AOAC-RI 112102

What kind of reference material is offered by R-Biopharm?

Certified Reference Materials (CRMs) for food allergen diagnostics are standardized materials with known concentrations of specific allergens. Even if there is a

huge demand and need, currently there are no CRMs available. However, R-Biopharm offers quality control materials for food allergen detection.

Quality control material offered by R-Biopharm:

SureFood® Quantard 40 Allergen

Art. No. S3301

13 allergens plus buckwheat with a concentration of 40 mg/kg; for qPCR quantification of allergens in food.

SureFood® Quantard 1ppm

Art. No. S3305

13 allergens plus buckwheat with a concentration of 1 mg/kg; for qPCR quantification of allergens in food.

Set of 3 processed Gliadin Assay Controls

Art. No. R7012

Three Gliadin assay controls: one below 10 ppm and two high positive homogenized snack samples; in cooperation with Trilogy® Analytical Laboratories.

Allergen testing

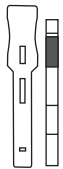
Which equipment is needed for the different test methods?

Depending on the analytical method, different equipment is needed. Consecutively, the graphic below combines the method with the necessary

equipment for three different lab sizes (point of care, medium sized lab, large lab).

Small laboratories

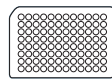
Lateral flow



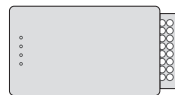
- bioavid lateral flow
- RIDA® QUICK
- RIDA®SMART BOX
- RIDA®SMART APP Allergen

Small to midsize laboratories

ELISA



- RIDASCREEN® ELISA



- MTP reader**
- RIDA®ABSORBANCE 96

qPCR manual



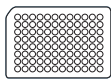
- SureFood® ALLERGEN



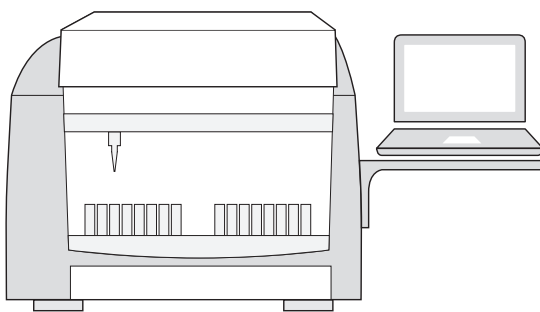
- RIDA®CYCLER

Large laboratories

ELISA



- RIDASCREEN® ELISA

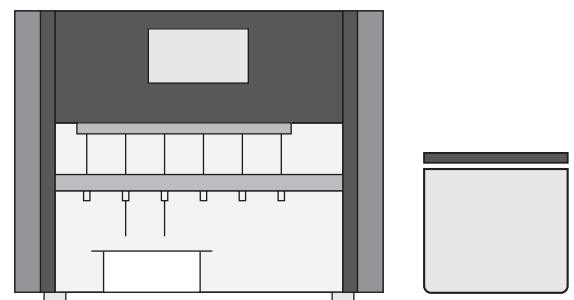


- ELISA analyzer**
- Bolt™/Thunderbolt®

qPCR automation



- SureFood® ALLERGEN



- TANBead Maelstrom™ 4800
- RIDA®CYCLER

Which equipment is available from R-Biopharm AG?

For ELISA test kits, R-Biopharm AG offers the RIDA® ABSORBANCE 96 Microtiter plate photometer and the ThunderBolt® 2-microtiter plate analyzer for RIDASCREEN® and RIDASCREEN® FAST ELISA test kits.

For real-time PCR, R-Biopharm offers the TANBead Maelstrom™ 4800 for automated DNA/RNA extraction and the RIDA® CYCLER for the real-time PCR analysis.



RIDA® ABSORBANCE 96

Absorbance reader

Innovative microtiter plate photometer including RIDASOFT® Win.NET software



ThunderBolt®

ELISA analyzer

Fully automated device for ELISA analysis in microtiter plate format
RIDASOFT® Win.NET software



TANBead Maelstrom™ 4800

Automated nucleic acid extraction system

For 16/32/48 samples

RIDA® CYCLER

Real-time PCR thermal cycler

For multiplex analyses & 48 samples

Allergen testing

Which method and product is best suited for your analytical need?

Depending on the customer's interests in allergen management, the laboratory equipment and the sample throughput, different customers have different analytical needs. Some analysts perform a hygiene control by swabbing the equipment after cleaning, others require quantitative information for one or more analytes in food samples. In some cases

qPCR is the method of choice, in other cases immune-based methods such as LFD or ELISA.

In order to determine the most suitable method and test kit for your demand, the most common needs and the corresponding recommended methods are listed in the table below.

Table 4: Overview of methods suitable for your need.

Demand	Recommended method	Explanatory note
Hygiene monitoring	ELISA; LFD	Analyzing swabs, CIP water and food for allergen contaminations with RIDA®QUICK or bioavid LFDs is fast and simple.
Multiplex testing	qPCR	SureFood® ALLERGEN 4plex kits allow simultaneous and individual detection of multiple allergens.
Qualitative allergen management	LFD; qPCR	Quick and easy qualitative analysis of allergen contamination using RIDA®QUICK and bioavid LFDs. Qualitative analysis by qPCR using SureFood® ALLERGEN and SureFood® ALLERGEN 4plex kits.
Quantitative allergen management	ELISA; qPCR	Food allergen diagnostic can be quantitatively performed by using RIDASCREEN®ELISA, RIDASCREEN®FAST ELISA or SureFood® ALLERGEN.
Simultaneous analysis of multiple samples	ELISA; qPCR	Up to 43 samples and up to 19 samples can be analyzed simultaneously by using RIDASCREEN® ELISA and RIDASCREEN®FAST ELISA respectively. Standardized sample and test procedure allow simultaneous analysis of multiple targets by using SureFood® ALLERGEN.
Analysis of milk and egg contaminations	ELISA; LFD	Immuno-based methods (RIDASCREEN®FAST ELISA, bioavid LFD) are the method of choice for egg and milk detection; qPCR cannot differentiate between egg and chicken or milk and cow.
Analysis of celery and fish	qPCR	qPCR is the method of choice. Many cross-reactivities are observed when using an immuno-based method for the detection of celery. There is no common epitope for fish detection in immuno-based methods.
Analysis of hydrolyzed or fermented samples	qPCR; ELISA	DNA is stable and can be extracted from highly processed foods, thus SureFood® ALLERGEN is suitable. After hydrolysis, peptides are detected and quantitatively determined by a competitive ELISA (RIDASCREEN® competitive). Common sandwich ELISA need two binding sides on the target analyte, therefore Sandwich ELISA are not applicable for hydrolyzed or fermented foods.
Confirmation of results	qPCR; ELISA	SureFood® ALLERGEN confirms ELISA results. RIDASCREEN® ELISA and RIDASCREEN®FAST ELISA confirm qPCR results or positive LFDs.
High sample throughput	ELISA; qPCR	Automation enables standardization and harmonization of your methods and reduces your handling time. The ThunderBolt® provides automated ELISA processing of two ELISA plates and thus of up to 192 samples simultaneously. The TANBead Maelstrom™ 4800 allows DNA extraction of 48 samples simultaneously. Each extracted sample can then be used for several qPCR runs and different targets.

Which testing approach is the right for me? We'll provide a few quick insights.

Medium sized contract labs



"As service lab we need to be able to provide our customers a wide range of different analytical test parameters. Depending on the customers need, we offer both, qPCR and ELISA.

As the reference method for gluten-free products, we use the RIDASCREEN®Gliadin in combination with Cocktail (patented) a lot. Nevertheless, if a customer does not need the official method and wants a faster and cheaper option, we also offer diagnostic with RIDASCREEN®FAST Gliadin.

Furthermore, due to the increasing

amount of incoming samples we decided to implement automated DNA extraction. SureFast® Mag PREP Food is an ideal DNA preparation kit, as it can be used for a variety of samples (plant & animal derived) as well as for bacterial enrichments. The automated extraction step reduces our hands on time and increases our productivity.

We are proud to offer all kind of possible detection methods to our customers and are happy to receive everything from one manufacturer."

Equipment

TANBead Maelstrom™

Test kits

ELISA

- RIDASCREEN® Gliadin: Cocktail (patented)
- RIDASCREEN®FAST Egg Protein
- RIDASCREEN®FAST Gliadin
- RIDASCREEN®FAST Milk
- RIDASCREEN®FAST Soya
- RIDASCREEN®FAST Mustard
- RIDASCREEN®FAST Lupin

qPCR

- SureFast® Mag PREP Food
- SureFood® ALLERGEN Soya
- SureFood® ALLERGEN Celery
- SureFood® ALLERGEN Mustard
- SureFood® ALLERGEN Lupin
- SureFood® ALLERGEN Pistachio

Other tests

- qPCR Animal identification
- qPCR Food pathogen detection
- qPCR GMO detection
- Enzymatic applications (sulfite)

Allergen testing

Manufacturer of gluten-free and GMO-free cereal products



“Gluten-free and GMO-free products are our focus. Our products are mainly based on corn and oats.

For a quick assessment, we immediately check incoming trucks with raw materials by using RIDA®QUICK Gliadin.

All incoming goods are also tested in our own lab. In former times, we sent our samples to an external contract lab, today we have results much faster due to our own company lab. Because we cannot

rule out contamination from wheat, rye and barley in oat samples, we use the RIDASCREEN® Total Gluten. For other samples we prefer using the ELISA RIDASCREEN® Gliadin test, which is the best validated gluten method and possesses a high analytical performance.

To ensure that our products are GMO-free, we also test the raw materials using the multiplex screening SureFood® GMO 4plex qPCR test systems.”

Equipment

RIDA®ABSORBANCE 96

ThunderBolt®

RIDA® CYCLER

Test kits

Lateral flow

- RIDA®QUICK Gliadin

ELISA

- RIDASCREEN®FAST Gliadin: Cocktail (patented)
- RIDASCREEN® Total Gluten

qPCR

- SureFood® GMO 4plex 35S/NOS/FMV/IAC
- SureFood® GMO 4plex BAR/ NPTII/PAT/CTP2:CP4 EPSPS

Governmental lab



"As a state lab, our major role is to verify the food product safety of our customers. As we are obliged to detect and identify all relevant allergens, we are using both technologies ELISA as well as qPCR.

We carry out screening of food product samples with ELISA, because many samples can be analyzed in little time. qPCR cannot differentiate between cow and milk or chicken and egg, we therefore evaluate the quantity of milk and egg residues in foods by using the RIDASCREEN® FAST Casein, RIDASCREEN®FAST Milk

and RIDASCREEN® Egg. The RIDASCREEN® Egg is a quite unique product on the market, since it detects native as well as processed egg proteins. qPCR is especially used for the detection and quantification of celery, as in this case immunoassays are not specific due to cross-reactions to closely related plants. Moreover, we carry out qPCR for confirmation testing in case of positive ELISA test results or in order to identify the gluten contamination source by using SureFood® ALLERGEN Cereals 4plex for example."

Equipment

RIDA®ABSORBANCE 96

Test kits

ELISA

- RIDASCREEN® Gliadin: Cocktail (patented)
- RIDASCREEN® Gliadin competitive
- RIDASCREEN®Fast Casein
- RIDASCREEN®Fast Soy
- RIDASCREEN®Fast Egg protein
- RIDASCREEN®Fast Egg
- RIDASCREEN®Fast Peanut
- RIDASCREEN®Fast Almond

qPCR

- SureFood® PREP Food
- SureFood® QUANTARD Allergen 40
- SureFood® ALLERGEN Mustard
- SureFood® ALLERGEN Celery
- SureFood® ALLERGEN Soy
- SureFood® ALLERGEN Gluten
- SureFood® ALLERGEN Cereals 4plex

Other tests

- qPCR Food pathogen detection
- qPCR Animal identification
- Vitamin analysis
- Enzymatic applications

Allergen testing

Multi-national sweet & chocolate company



"We produce a variety of confectionary products and have to make sure to be compliant with the allergen regulations and declaration rules.

Directly at first stage, we control incoming raw material with lateral flow tests. With this, we can quickly test for potential contamination sources. We also perform final product testing in order to offer safe end products for the consumer. We mainly use ELISA in our own labs around the

world. For some specific needs such as mustard detection and for confirmation, we will implement qPCR in our labs too.

For our hygiene control, we use the lateral flow tests which give us a fast result and thus feedback about the cleaning process.

The combination of all methods will lead to a more efficient workflow and will save time in releasing incoming raw materials and final products."

Equipment

ThunderBolt®

Test kits

ELISA

- RIDASCREEN® Peanut
- RIDASCREEN®Fast Hazelnut
- RIDASCREEN®Fast Milk
- RIDASCREEN®Fast Egg Protein
- RIDASCREEN®Fast Almond
- RIDASCREEN®Fast Gliadin
- RIDASCREEN®Fast Soy
- RIDASCREEN®Fast Sesame

qPCR

- SureFood® ALLERGEN Mustard

Other tests

- Mycotoxin analysis



Allergens – R-Biopharm product overview

	RIDASCREEN®	RIDA®QUICK/bioavid	SureFood®
	ELISA	Lateral flow tests	Real-time PCR
Gliadin/Gluten			
Gliadin/Gluten	●*	●	●
Gliadin/Gluten fragments	●*		
Egg			
Egg	●*	●●	
Lysozyme	●*		
Milk			
β-Lactoglobulin	●*		
β-Lactoglobulin fragments	●		
Casein	●*		
Milk	●*	●	
Nuts and similar			
Almond	●*	●●	●●
Brazil nut		●	●●
Cashew kernel	●	●	●●
Coconut		●●	
Hazelnut	●*	●●	●●
Macadamia		●	●●
Peanut	●*	●●	●●
Pecan			●●
Pine			●
Pistachio		●	●●
Shea nut			●
Walnut		●	●●
Seafood			
Crustacean	●*	●	●
Fish			●
Molluscs			●
Various			
Apricot			●
Buckwheat			●
Celery			●●
Insects			●
Lupine	●		●
Mustard	●*	●●	●●
Oat			●
Sesame	●*	●●	●
Soya	●*	●	●●

- * ELISA is suitable for automation
- Also available with hook line
- SureFood® ALLERGEN 4plex Kits

Other parameters (like histamines, glutamic acid, lactose and sulfites), that are often diagnosed in connection with allergies, can be found in the corresponding other chapters of our product catalogue.

R-Biopharm AG

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