R-Biopharm AG

RIDASCREEN®FAST Soya

Art. No. R7102

Validation Report

10/2012

www.r-biopharm.com

RIDASCREEN®FAST Soya Art. No. R7102

General

RIDASCREEN®FAST Soya is a **sandwich enzyme immunoassay** for the quantitative analysis of soya proteins in untreated and processed food and beverages.
The test is able to detect specifically denaturized soya proteins from raw soybeans, flour, protein concentrates and various products made from soybeans. The testing of soya protein isolates and hydrolyzates is recommended with restrictions.
In studies most of the soybean allergic persons in Europe are sensitized to the subunits of glycinin and ß-conglycinin. The RIDASCREEN®FAST Soya ELISA is able to detect e.g. the subunits of these major allergens beside others in processed and unprocessed food thanks to its specific antibodies and its special sample preparation.

Soya has become an important protein source as a cheap alternative e.g. as replacement for skim milk in formulas for young mammals. Thus, soya is widely used as substitute for proteins from animal sources (e.g. in tofu, soya milk or soya yoghurt). Besides the high protein content, soybeans are also rich in fat (approx. 20 %) and can be used for the productions of oils and fats.
Beside nutrition, soya protein is widely used in food technology to improve functional properties such as foaming, gelling, or emulsification during processing.
The increasing use of soya as a food may increase the prevalence of soya allergies.

Several **soya proteins** can cause allergic reactions. The symptoms range from mild rashes like dermatitis, diarrhea and nausea to life threatening systemic anaphylaxis.

Soybeans (mature seeds, raw) contain approx. 35 % proteins and comprises 10 % albumins and 90 % seed storage globulins. The globulins can be fractionated on the basis of their coefficient of sedimentation. Thus, the main protein fractions are named 11S globulin (glycinin; Gly m6, 350 kDa), representing 40 % of the total storage proteins and the 7S globulin (ß-conglycinin, Gly m5, 180 KDa) representing 30 % of the total seed storage protein (Sampson & McCaskill 1985, Garcia et al. 1997). **Glycinin** is present as a hexamer and consists of six pairs of non-identical subunits. Each subunit consists of an acidic (34 kDa) and a basic polypeptide (20 kDa) linked via a disulfide bond.
ß-Conglycinin is a trimeric glycoprotein, composed of three subunits, α- (72 kDa), α'- (76 kDa) and ß-subunit (53 kDa) which are associated via hydrophobic interactions. *Holzhauser et al.* 2008 showed that all subunits of Gly m5 and Gly m6 are IgE-reactive (53 % of the study subjects). The IgE reactivity was measured before in 30 sera from subjects with a positive double-blind, placebo-controlled soybean challenge (*Ballmer-Weber & Holzhauser et al.* 2007). Therefore an IgE binding to Gly m5 and Gly m6 was found in 86 % subjects with anaphylaxis to soya.
At date 16 IgE binding soya proteins have been described but not all of them are well characterized.

A further major soybean allergen has been described in the **thiol protease** Gly m Bd 30k. Linear epitopes inducing an IgE reaction have been reported (*Helm et al.* 1998).

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Trypsin inhibitors like the Kunitz soybean trypsin inhibitor (*Kunitz* 1947) are minor allergens whose activity is usually destroyed in most cases by heating of the native soybean. Up to 20 % of the activity can remain in heat treated soya flours.

The hull proteins Gly m1 and Gly m2 contained in soybean dust induces an inhalation allergy also called occupational or environmental soybean allergy.
In adults an allergy to Gly m3 and Gly m4 is often caused by a cross sensitization with the birch pollen allergen Bet v1. In heated and highly processed food such as soya sauce, soybean oil, roasted soybeans but also in cold-pressed soybean oil a small content of Gly m4 was detectable. The allergenicity of the 2S albumin in not clear yet.

Often soy allergic people react also to other leguminosae like beans or peanut and some also reacts to cow's milk or house dust mite.

Dose-response experiments showed that different amounts of soya protein trigger the food allergy to soya. Objective allergic reactions may appear at about 5 mg soya protein.

Test Principle

The basis of the sandwich ELISA is an antigen-antibody reaction. Detailed information is contained in the test kit insert.

Sample preparation

The sample extraction is carried out using the RIDASCREEN® Allergen Extraction Buffer in combination with Extraction solution 2 as described in the test kit insert.

Calibration Curve

A typical standard curve for RIDASCREEN®FAST Soya is shown in appendix 1. The result is expressed as mg/kg (ppm) soya proteins. To create the calibration curve a cubic spline function is recommended.

Specificity

The RIDASCREEN®FAST Soya ELISA contains monoclonal antibodies detecting especially highly heated, denatured soya proteins and the resulting allergic subunits. The cross reactivity to heated glycinin is 408 %, to heated ß-conglycinin 7.3 % and to heated trypsin inhibitor about 0.46 %.The soy flour (40 % protein) standard curve is calibrated to soya protein. There is no cross reaction to peanut, lentil, pea, lupine and milk or egg proteins. A mild cross reaction to some leguminosae (beans) was measured (see cross reactivity).

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Sensitivity

Limit of Detection (LOD)

The **Limit of Detection** or the lowest detectable concentration of soya proteins in the RIDASCREEN®FAST Soya ELISA that can be distinguished from zero matrices was determined to be 0.31 mg/kg (ppm) soya proteins (see table 1).
For the determination of the LOD three different soya free matrices have been extracted 10 times, measured in duplicates. The LOD is given as the mean plus 3 x standard deviation.

Table 1 Determination of the Limit of Detection (LOD)

Sample	Standard 1 (OD*)	Standard 2 (OD)	Mean of samples** (OD*)	Standard deviation (SD)	Mean + 3x SD (OD)	LOD (mg/kg)
Bread bakery mixture	0.061	0.333	0.078	0.006	0.096	0.33
Sausage	0.065	0.385	0.071	0.005	0.085	0.30
Chocolate	0.058	0.362	0.071	0.005	0.085	0.31
Mean value						**0.31**

*OD = optical density
**mean of 10 extractions, each measured in duplicates

Limit of Quantification (LOQ)

The **Limit of Quantification** or the lowest concentration that can be determined in a sample with acceptable precision (repeatability) and accuracy under the stated conditions of the test was proofed to be 2.5 ppm soya protein (dilution factor of 100 included).

The LOQ is proofed by showing the range of samples spiked at the level of standard 2 (2.5 mg/kg soya protein). Each sample was extracted 10 times and measured in duplicates. The measured sample ranges are similar to the range of standard 2 (see table 2).

Table 2 Proof of the Limit of Quantification (LOQ) with spiked samples in comparison to standard 2

Sample	Measured concentration** (mg/kg)	Standard deviation (SD)	CV* (%)	LOD (mg/kg)
Standard 2	**2.5**	**0.042**	**1.7**	**2.4 - 2.6**
Bread bakery mixture	2.9	0.106	3.6	2.6 - 3.3
Margarine	2.5	0.094	3.7	2.2 - 2.8
Buffer	2.7	0.130	4.8	2.3 - 3.1

*OD = optical density
**mean of 10 extractions, each measured in duplicates

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Precision (scatter of replicate readings around their mean value)
To determine the reproducibility and repeatability of the RIDASCREEN®FAST Soya ELISA intra-assay and inter-assay coefficients were calculated.

Variance of the ELISA

Intra-Assay Variation (repeatability)

Within run variation was calculated by measuring the standards in one assay run (n=6). The within assay variation over the standard curve is 4.6 % (data not shown). The average intra-assay variation of spiked samples has been found at 3.7 % with a mean recovery of 99 % (see table 3).

Table 3 Determination of the Intra-Assay Variation (repeatability) of spiked samples (n=6)

Sample	Target soya protein concentration (mg/kg)	Concentration measured (mg/kg)	Standard deviation (SD)	CV* (%)	Recovery (%)
Cookie	0.0	< LOQ			
Margarine 1	5.0	4.6	0.242	5.3	92
Margarine 2	8.0	8.5	0.180	2.1	106
Mean value				**3.7**	**99**

*CV = coefficient of variation

Inter-Assay Variation (reproducibility)

Between run variation was determined by repeated measurements of the standard curves from different test kits of one production batch performed by three technicians (n=3 runs). In each run duplicates of samples and standards have been measured. The mean between assay variance for the RIDASCREEN®FAST Soya is 8.7 % over the standard curve (data not shown). The inter-assay variance of one negative and two spiked food samples is 4.1 % with a mean recovery of 99 % (see table 4).

Table 4 Determination of the Inter-Assay Variation (reproducibility) of spiked samples

Sample	Target soya protein concentration (mg/kg)	Concentration measured (mg/kg)	Standard deviation (SD)	CV* (%)	Recovery (%)
Cookie	0.0	< LOQ			
Margarine 1	5.0	4.7	0.088	1.8	95
Margarine 2	8.0	8.2	0.525	6.4	103
Mean value				**4.1**	**99**

*CV = coefficient of variation

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Cross reactivity

Approximately 100 compounds were evaluated. A cross reactivity was observed for several foods as can be seen in table 5a and 5b. A slight cross reactivity of 0.0017 % consists of leguminosae of the tribe Phaseoleae (various species of beans) and to leguminosae of the genus Vicia faba group (0.0003 %).

Table 5a Overview of different foods tested for cross reactivity at a hundred percent level. All samples have been extracted according to the leaflet using Extraction solution 2 combined with Allergen Extraction buffer.

Sample description	Optical density	Soya protein (mg/kg)
Nuts		
Std 1	0.079	
Std 2	0.241	
Cashew	0.055	< LOQ
Walnut	0.054	< LOQ
Almond	0.049	< LOQ
Macadamia nut	0.088	< LOQ
Pecan nut	0.077	< LOQ
Pistachio nut	0.069	< LOQ
Coconut	0.092	< LOQ
Brazil nut	0.075	< LOQ
Hazelnut raw	0.073	< LOQ
Cereals		
Std 1	0.079	
Std 2	0.241	
Wheat	0.068	< LOQ
Barley	0.078	< LOQ
Rye	0.077	< LOQ
Rice flour	0.079	< LOQ
Oat meal	0.080	< LOQ
Maize flour	0.128	< LOQ
Millet	0.061	< LOQ
Buckwheat flour	0.131	< LOQ
Spelt	0.071	< LOQ
Quinoa	0.058	< LOQ
Kamut	0.071	< LOQ
Amaranth	0.090	< LOQ

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Sample description	Optical density	Soya protein (mg/kg)
Beans/lentils/peas		
Std 1	0.078	
Std 2	0.241	
Horse bean (*Vicia faba*)	**0.680**	**5.9**
Scarlet runner bean (*Phaseolus coccineus*)	**2.620**	**> Std 5**
Bush bean (*Phaseolus vulgaris*)	**1.013**	**8.8**
Lima beans (*Phaseolus lunatus*)	**1.197**	**10.5**
Mung bean (*Vicia radiata*)	**3.147**	**> Std 5**
Azuki bean (*Vigna angularis*)	**3.000**	**> Std 5**
Kidney been	**1.483**	**13.43**
White bean	**1.429**	**12.86**
Pinto bean	**1.316**	**11.7**
Green pea	**0.599**	**6.18**
Wrinkled pea (*Pisum sativum*)	0.167	< LOQ
Winter pea (*Vicia faba*)	0.169	< LOQ
Pannonic hairy vetch (*Vicia sp.*)	0.117	< LOQ
Brown lentil (*Lens culinaris*)	0.140	< LOQ
Peanut (raw)	0.075	< LOQ
Peanut (roasted)	0.095	< LOQ
Chickpea (*Cicer arietinum*)	0.104	< LOQ
Fenugreek (*Trigonella foenum-graecum*)	0.184	< LOQ
Red clover (*Trifolium pratense*)	0.123	< LOQ
Lupinus albus	0.097	< LOQ
Lupinus luteus	0.084	< LOQ
Lupinus angustifolius	0.066	< LOQ
Seeds		
Std 1	0.079	
Std 2	0.241	
Sunflower kernels	0.066	< LOQ
Sesame	0.093	< LOQ
Pumpkin kernels	0.053	< LOQ
Apricot stones	0.050	< LOQ
Pine seed	0.109	< LOQ
Poppy seeds	0.078	< LOQ
Chestnut	0.076	< LOQ
Linseed	0.14	< LOQ
Rape seed	0.076	< LOQ

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Sample description	Optical density	Soya protein (mg/kg)
Spices		
Std 1	0.055	
Std 2	0.258	
Mustard seeds (yellow)	0.072	< LOQ
Mustard seeds (black-brown)	0.083	< LOQ
Celery seed	0.173	< LOQ
Celery powder	0.063	< LOQ
Celery leafs	0.062	< LOQ
Fennel seed	0.066	< LOQ
Anise seed	0.080	< LOQ
Caraway	0.061	< LOQ
Black caraway	0.062	< LOQ
Cumin	0.061	< LOQ
Curcuma	0.080	< LOQ
Chili powder	0.068	< LOQ
Paprika powder, sweet	0.069	< LOQ
Paprika powder, hot	0.061	< LOQ
Cinnamon	0.060	< LOQ
Cloves	0.072	< LOQ
Pepper, white	0.064	< LOQ
Pepper, hot	0.062	< LOQ
Various		
Std 1	0.055	
Std 2	0.258	
Skim milk powder	0.080	< LOQ
Bovine gelatin	0.061	< LOQ
Porcine gelatin	0.057	< LOQ
Fish gelatin	0.055	< LOQ
Egg lecithin	0.077	< LOQ
Soy lecithin	**3.211**	**77***
Soy oil	0.065	< LOQ
Apricots kernel oil	0.064	< LOQ
Wine (red)	0.062	< LOQ
Wine (white)	0.063	< LOQ
Kiwi	0.055	< LOQ
Cocoa	0.075	< LOQ
Coffee	0.134	< LOQ
Margarine	0.063	< LOQ
Ketchup	0.067	< LOQ
Sugar	0.074	< LOQ
Ovalbumin	0.062	< LOQ
Whole egg powder	0.056	< LOQ
Foetal calf serum	0.051	< LOQ
Bovine serum albumin	0.051	< LOQ
Linseed	0.14	< LOQ
Rape seed	0.076	< LOQ

*protein contaminated

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Table 5b Cross reactivity of some leguminosae

	mean CR* (%)
Scarlet runner bean (*Phaseolus coccineus*)	0.00146
Mung bean (*Vigna radiata*)	0.00534
Azuki bean (*Vigna angularis*)	0.00104
Bush bean (*Phaseolus vulgaris*)	0.00029
Winter bean (*Vicia faba*)	0.00030
Lima bean (*Phaseolus lunatus*)	0.00027
Linseed	0.00006

*CR = cross reactivity

Recovery of unprocessed spiked samples and processed food

Unprocessed food matrices measured well below the LOQ have been spiked with a soya flour solution (40 % protein). For the recovery of processed food, nut cake dough was mixed with soya flour (40 % protein) afterwards baked under various conditions in an oven. Minced meat has been mixed with different amounts of soya flour, afterwards processed under constant baking conditions (20 min at 150 °C). The samples were extracted according to the test kit insert. A mean recovery over the unprocessed spike samples were found at 98 % (see table 6a) and for the processed food at 91 % (see table 6b).

Table 6a Recovery (%) of spiked samples and processed food

Sample description	Target soya protein concentration (mg/kg)	Concentration measured (mg/kg)	Recovery (%)
Spike control	2.5 5 10 20	2.2 4.6 9.2 18.2	90 92 92 91
Nut nougat cream	2.5 5 10 20	2.3 4.3 9.0 18.0	92 86 90 90
Bakery mixture	2.5 5 10 20	2.7 5.4 11.7 23.0	109 109 117 115
Margarine	2.5 5 10 20	2.3 4.6 9.6 19.1	92 92 96 95
Sausage	2.5 5 10 20	2.2 4.7 9.1 19.4	86 94 91 97
Ketchup	2.5 5 10 20	2.5 5.1 10.0 24.2	100 102 100 121
Mean			**98**

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Table 6b Recovery (%) of processed food

Sample description	Target soya protein concentration (mg/kg)	Concentration measured (mg/kg)	Recovery (%)
Hazel nut cake dough + 1 % soya flour	4000	3255	81
Hazel nut cake + 1 % soya flour 10'/200°C	4000	2138	53
Hazel nut cake + 1 % soya flour 8'/200°C	4000	2745	69
Hazel nut cake + 1 % soya flour 15'/180°C	4000	2132	53
Hazel nut cake + 1 % soya flour 10'/180°C	4000	2419	60
Hazel nut cake + 1 % soya flour 20'/150°C	4000	2820	70
Hazel nut cake + 1 % soya flour 15'/150°C	4000	2530	63
Hazel nut cake + 1 % soya flour 10'/150°C	4000	2801	70
Minced meat raw + 1 % soya flour	4000	3891	97
	4000	3326	83
	4000	4506	113
Minced meat raw + 0.1 % soya flour	400	350	88
	400	372	93
	400	377	94
Minced meat raw + 0.005 % soya flour	20	22.0	110
	20	24.3	121
	20	25.0	125
Minced meat heated** + 1 % soya flour *	8000	9261	116
	8000	8323	104
	8000	8977	112
Minced meat heated + 0.1 % soya flour *	800	671	84
	800	725	91
	800	703	88
Minced meat heated + 0.005 % soya flour *	40	45.4	113
	40	44.3	111
	40	44.6	111
Mean			**91**

* approx. 40 - 50 % loss of water and fat is included before calculating of the recovery
**20 min/ 150 °C

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Samples from the market

Various samples have been collected from the market and measured (see table 7). Some samples are declared using the precautionary labeling “may contain soya”. Eleven of the declared samples contained soya higher than the LOQ. Six non declared samples contained soya higher than the LOQ (see table 7).

Table 7 Measurement of samples from the market

Declaration	Dilution factor	Soya declared	Optical density	Soya protein (mg/kg)
Waffle with soya flour and whey powder	1000	yes	0.628	5946
Whole meal bread	1		0.186	< LOQ
Flatbread with lupine flour	1		0.049	< LOQ
Crisp bread. gluten and lactose free	1		0.076	< LOQ
Sour apple bar	1		0.105	< LOQ
Nut cake dough	1		0.063	< LOQ
Biscuit	1		0.063	< LOQ
Noodles - Original Japanese roasted noodles, chili	1	yes	0.064	< LOQ
Soya sauce - Original Japanese roasted noodles, chili	1	yes	0.060	< LOQ
Spices - Original Japanese roasted noodles, chili	1	yes	0.059	< LOQ
Vegetables - Original Japanese roasted noodles, chili	1	yes	0.070	< LOQ
Noodles - Instant noodle snack duck	1	yes	0.670	**7.13**
Spices - Instant noodle snack duck	1	yes	0.086	< LOQ
Vegetable pan meal - chicken	1		0.063	< LOQ
Liver sausage	1		0.061	< LOQ
Ham sausage	1		0.060	< LOQ
Nurnberger grilled sausage	1		0.064	< LOQ
Plant crème , soya based	1	yes	0.058	< LOQ
Half fat margarine with starch	1	yes	0.061	< LOQ
Half fat margarine with yoghurt	1		0.058	< LOQ
Margarine, salted	1		0.061	< LOQ
Half fat margarine	1		0.056	< LOQ
Almond cookies	1		0.076	< LOQ
Cereals	1		0.058	< LOQ
Whole meal zwieback	1		0.069	< LOQ
Egg spaghetti	1		0.278	**2.59**
Bakery mixture, gluten free	1		0.077	< LOQ
Egg noodles	1		0.088	< LOQ
Shortbread biscuits	2000	yes	0.415	**10916**
Digestive biscuits	2000	yes	0.415	**10916**
Chocolate	200	yes	0.303	**822**
Chocolate chip cookie	2000	yes	0.626	**16009**
Chocolate cookies	200	yes	0.765	**1914**
Maize flour, contaminated	1		0.070	< LOQ
Creme waffle, gluten free	2000	yes	0.868	**21371**
Sunflower kernel, powder	1		0.080	< LOQ

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Declaration	Dilution factor	Soya declared	Optical density	Soya protein (mg/kg)
Egg substitute 1	2		1.786	**47**
Egg substitute 2	1		0.218	< LOQ
Semolina pudding	1		0.068	< LOQ
Waffle with cocoa, gluten free	2000	yes	0.676	**17155**
Single rice, cereal for baby	1		0.329	**3.07**
Honeyroasted rice	1		0.064	< LOQ
Teff flour mixture	200		0.476	**1242**
Bread	1		0.130	< LOQ
Hypoallergenic baby food	1		0.059	< LOQ
Brownies bakery mixture	1		0.063	< LOQ
Cracker	1		0.074	< LOQ
Maize Snack	1		0.086	< LOQ
Milk free, soy containing infant food 1	2000	yes	0.605	**15521**
Milk free, soy containing infant food 2	200	yes	1.227	**2946**
Pizza bakery mixture, gluten free	1		1.618	**17.38**
Rice flakes	1		0.064	< LOQ
Bakery mixture chestnut bread	1		0.722	**7.17**
Flour mixture	1		0.216	< LOQ

Stability of the Test

The stability of the test is routinely checked by R-Biopharm's quality assurance laboratory after defined storage intervals. Test kits are stored in a cold room at temperatures of 2 - 8 °C (35 - 46 °F). Before testing the kit components are equilibrated to room temperature (20 - 25 °C / 68 - 77 °F). Real time stability of the test is regularly controlled according to the total quality management schedule of the company.

Conclusion

With the new RIDASCREEN®FAST Soya a sensitive ELISA is available which allows a quantitative and fast determination of soya proteins in processed and unprocessed food. The results are expressed as mg/kg soya proteins.

For further question or information please contact R-Biopharm AG directly:
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List of references

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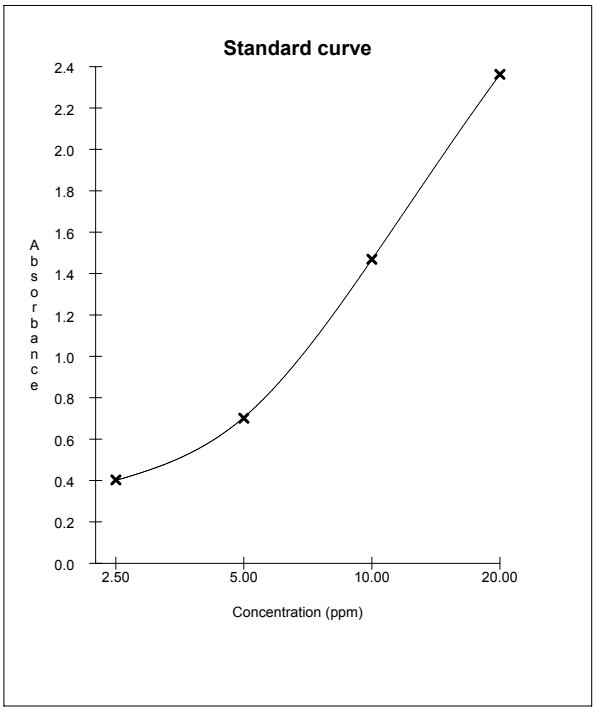
Appendix

QUALITY ASSURANCE CERTIFICATE

RIDASCREEN® FAST Soya

Art. No.: R7102 Lot: 13382 Expiry: 2013-10

R-Biopharm AG, Darmstadt, Germany certifies that this batch has been approved by the Quality Assurance Department and conforms with specifications



Standards

Std. Conc.(ppm) CV(%)	n mean
Std1 0.00 4.6	4 0.047
Std2 2.50 2.9	4 0.403
Std3 5.00 8.0	4 0.701
Std4 10.00 5.8	4 1.469
Std5 20.00 3.3	4 2.363

	Lot No.	Expiry
Microwell plate	15222	2013-11
Standards	11352	2013-10
Conjugate	11352	2013-10
Red Chromogen Pro	13451	2014-02
Stop solution	11102	2017-02
Washing buffer	12461	2014-04
Allergen Extr. buffer	12481	2014-04
RIDA® Extraction Solution 2	13292	2014-06

Please note:

The absorbance for the standards may decrease during the shelf life of the kit. The general shape of the curve will remain similar, while the slope might change slightly. Furthermore refer to product leaflet 8. Indication of instability or deterioration of reagents.

sign.: Edda Rohm
Quality Assurance Representative

Date: 19.09.2012

Remark: This document has been created electronically and is therefore valid without a signature.

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