# RIDASCREEN®FAST Peanut

## Art. No. R6202

**Product information**

# RIDASCREEN®FAST Peanut

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## General

Although peanut (*Arachis hypogaea*) belongs to the legumes and not to the nuts, its taste and its usual consumption is similar to tree nuts. So to speak, peanut is one of the most popular “nuts” worldwide. Peanuts are very common ingredients of chocolate products and are also consumed as snacks and in bakery products such as cookies.
Peanut is also one of the five foods which are the most frequent cause of food allergies. Allergies against peanuts are among the most severe allergies and can lead to life-threatening anaphylactic shocks.
Consumption of probably less than a milligram of peanut can induce allergic reactions in very sensitized individuals. Therefore, labelling of peanut containing products is very important and compulsory in the USA and the European Union as well as in many other countries.
The main threat for sensitized individuals is contamination of peanut in food which is intended to be peanut-free (e.g. peanut traces in chocolate which should not contain peanuts). Therefore, it is important to test foods which may have been contaminated during their production process for the presence of unintended traces of peanuts.

## Sample preparation

**Extraction**
The sample extraction is carried out as described in the instruction for use. For tannin and polyphenol containing samples such as spices and chocolate, the addition of skim milk powder to the sample is recommended.
Additionally, the addition of skim milk powder is also recommended for the extraction of ice cream and caramel, as described in the instruction for use.

## Spiking instruction

Detailed information on spiking of samples can be found in appendix 2.

## Calibration curve

The basis of the sandwich ELISA is an antigen-antibody reaction. Detailed information can be found in the instructions for use. As a calibrator, NIST peanut butter 2387 with a protein content of 22.2 % is used. The result is expressed as mg/kg peanut.
A typical standard curve is shown in appendix 1. To generate the calibration curve, a cubic spline function is recommended.

## Specificity

The antibodies specifically detect antigens from peanuts, mainly allergenic proteins such as Ara h 1 to Ara h 3. Cross-reactivities to green peas, lentils and fenugreek were observed.
To recheck the cross-reactivity we recommend using a non-immunoassay method such as PCR, SureFood® ALLERGEN ID.

To generate the data presented in this validation report, equipment as described in appendix 3 was used.

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## Sensitivity

### Limit of Detection (LOD)

Different zero matrices have been extracted 10 times each to estimate the **Limit of Detection**. A second order polynomial function was used for the extrapolation of the standard curve to calculate from the OD values of the zero matrices to the respective allergen concentration. The **Limit of Detection** was then estimated by the mean of these concentrations plus 3 times standard deviation (dilution factor for sample preparation is included).
The **Limit of Detection** is matrix-dependent.

**Table 1:** Estimation of the Limit of Detection (LOD).

| Matrix | Standard 1 (OD*) | Standard 2 (OD*) | Mean of samples (OD*) | Mean (mg/kg) | Standard deviation mean (mg/kg) | Mean + 3x standard deviation (mg/kg) |
|---|---|---|---|---|---|---|
| Chocolate | 0.212 | 0.703 | 0.220 | 0.04 | 0.05 | 0.19 |
| Cookies | 0.212 | 0.703 | 0.198 | -0.07 | 0.08 | 0.16 |
| Ice cream | 0.212 | 0.703 | 0.186 | -0.12 | 0.05 | 0.04 |
| Mean value (mg/kg) | | | | | | 0.13 |

* OD = optical density

### 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 mg/kg (dilution factor included). The LOQ is proofed by showing the range of samples spiked at the level of standard 2. Each sample was extracted 10 times and measured in duplicates. The measured sample ranges are similar to the range of standard 2, measured 10 times. All measured coefficients of variation are within an acceptable range showing that samples with a concentration of 2.5 mg/kg can be measured with good precision and accuracy.

**Table 2:** Determination of the Limit of Quantification by extracting spiked samples ten times.

| Sample | Measured concentration (mg/kg) | Standard deviation (SD) | Coefficient of variation (%) |
|---|---|---|---|
| Standard 2 (n=10) | 2.38 | 0.24 | 9.9 |
| Chocolate (n=10) | 2.24 | 0.11 | 5.1 |
| Cookie (n=10) | 2.98 | 0.16 | 5.3 |

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## Variance of the ELISA

To determine the reproducibility and repeatability of the ELISA, intra-assay and inter-assay coefficients were calculated.

**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 was 8.1 % (OD-value based, data not shown). The Intra-Assay Variation of spiked samples has been found in a good range.

**Table 3:** Determination of the Intra-assay variation (repeatability) of spiked samples.

| Matrix | Expected concentration (mg/kg) | Concentration found (mg/kg) | Coefficient of variation (%) | Recovery (%) |
|---|---|---|---|---|
| Cookie | 5.0 | 4.9 | 5.2 | 98 |
| Chocolate | 20.0 | 21.1 | 10.9 | 104 |
| Ice cream | 0 | <LOQ | | |
| Mean value | | | 8.0 | 101 |

**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 was 7.5 % over the standard curve (OD-value based, data not shown). The Inter-Assay Variance of spiked food samples has been found in a good range.

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

| Matrix | Expected concentration (mg/kg) | Concentration found (mg/kg) | Coefficient of variation (%) | Recovery (%) |
|---|---|---|---|---|
| Cookie | 5.0 | 5.4 | 1.6 | 108 |
| Chocolate | 200 | 20.2 | 1.6 | 101 |
| Ice cream | 0 | < LOQ | | |
| Mean value | | | 1.6 | 104 |

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## Cross reactivity

Food samples from different origins were evaluated as a pure commodity (100 % level) for their cross reactivity in the ELISA. Samples found well below the LOQ showed no cross reactivity. Other samples indicated in bold letters showed a signal over LOQ, for these samples a cross reactivity was demonstrated.

**Table 5:** Overview of different foods tested for cross reactivity at a hundred percent level. All samples have been extracted according to the instruction for use.

| Sample description | OD Standard 1* | OD Standard 2* | OD Sample | Result (mg/kg) |
|---|---|---|---|---|
| **Cereals/pseudocereals** | | | | |
| Amaranth | 0.206 | 0.659 | 0.149 | <LOQ |
| Barley | 0.206 | 0.659 | 0.185 | <LOQ |
| Buckwheat flour | 0.206 | 0.659 | 0.381 | <LOQ |
| Corn flour | 0.206 | 0.659 | 0.106 | <LOQ |
| Kamut | 0.206 | 0.659 | 0.140 | <LOQ |
| Millet flour | 0.206 | 0.659 | 0.087 | <LOQ |
| Oat flour | 0.206 | 0.659 | 0.091 | <LOQ |
| Quinoa | 0.206 | 0.659 | 0.076 | <LOQ |
| Rice flour | 0.206 | 0.659 | 0.075 | <LOQ |
| Rye | 0.23 | 0.637 | 0.307 | <LOQ |
| Spelt | 0.23 | 0.637 | 0.279 | <LOQ |
| Teff flour | 0.228 | 0.572 | 0.169 | <LOQ |
| Wheat | 0.228 | 0.572 | 0.232 | <LOQ |
| **Beans/lentils/peas** | | | | |
| Chick peas | 0.228 | 0.572 | 0.268 | <LOQ |
| Green bean | 0.244 | 0.696 | 0.299 | <LOQ |
| Green peas | 0.187 | 0.621 | 0.840 | **3.65** |
| Kidney beans | 0.228 | 0.572 | 0.276 | <LOQ |
| Lentils | 0.187 | 0.621 | 0.948 | **4.35** |
| Lima beans | 0.228 | 0.572 | 0.174 | <LOQ |
| Pinto beans | 0.228 | 0.572 | 0.363 | <LOQ |
| Soy milk | 0.228 | 0.572 | 0.214 | <LOQ |
| Soya flour | 0.230 | 0.637 | 0.635 | <LOQ |
| White beans | 0.134 | 0.484 | 0.143 | <LOQ |
| Soya flour | 0.258 | 0.337 | 0.214 | <LOQ |
| White beans | 0.258 | 0.337 | 0.212 | <LOQ |
| **Seeds/stones/kernel** | | | | |
| Linseed | 0.228 | 0.572 | 0.206 | <LOQ |
| Pine kernel | 0.228 | 0.572 | 0.122 | <LOQ |
| Poppy seeds | 0.235 | 0.718 | 0.233 | <LOQ |
| Pumpkin kernels | 0.228 | 0.572 | 0.287 | <LOQ |
| Rape seed | 0.235 | 0.718 | 0.223 | <LOQ |
| Sesame | 0.235 | 0.718 | 0.232 | <LOQ |
| Sunflower kernels | 0.235 | 0.718 | 0.174 | <LOQ |

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| Sample description | OD Standard 1* | OD Standard 2* | OD Sample | Result (mg/kg) |
|---|---|---|---|---|
| **Nuts** | | | | |
| Brazil nut | 0.235 | 0.718 | 0.170 | <LOQ |
| Cashew | 0.235 | 0.718 | 0.341 | <LOQ |
| Cashew (roasted) | 0.235 | 0.718 | 0.208 | <LOQ |
| Chestnut | 0.235 | 0.718 | 0.180 | <LOQ |
| Macadamia | 0.235 | 0.718 | 0.267 | <LOQ |
| Pecan nut | 0.235 | 0.718 | 0.182 | <LOQ |
| Pistachio nut | 0.235 | 0.718 | 0.199 | <LOQ |
| Walnut | 0.235 | 0.718 | 0.211 | <LOQ |
| Walnut (roasted) | 0.235 | 0.718 | 0.204 | <LOQ |
| **Spices** | | | | |
| Aniseed | 0.235 | 0.718 | 0.266 | <LOQ |
| Basil | 0.214 | 0.590 | 0.493 | <LOQ |
| Caraway | 0.214 | 0.590 | 0.195 | <LOQ |
| Cardamon | 0.214 | 0.590 | 0.152 | <LOQ |
| Celery (powder) | 0.214 | 0.590 | 0.187 | <LOQ |
| Chilli | 0.214 | 0.590 | 0.211 | <LOQ |
| Cinnamon | 0.214 | 0.590 | 0.213 | <LOQ |
| Cloves | 0.198 | 0.682 | 0.320 | <LOQ |
| Cumin | 0.214 | 0.590 | 0.194 | <LOQ |
| Curcuma | 0.198 | 0.682 | 0.168 | <LOQ |
| Curry | 0.214 | 0.590 | 0.271 | <LOQ |
| Fennel seed | 0.214 | 0.590 | 0.185 | <LOQ |
| Fenugreek | 0.198 | 0.682 | 0.779 | **2.88** |
| Garlic | 0.214 | 0.590 | 0.531 | <LOQ |
| Ginger | 0.214 | 0.590 | 0.208 | <LOQ |
| Marjoram | 0.141 | 0.544 | 0.166 | <LOQ |
| Mustard seeds (black-brown) | 0.214 | 0.590 | 0.173 | <LOQ |
| Mustard seeds (yellow) | 0.141 | 0.544 | 0.147 | <LOQ |
| Nutmeg | 0.141 | 0.544 | 0.112 | <LOQ |
| Onion (powder) | 0.141 | 0.544 | 0.120 | <LOQ |
| Paprika (sweet) | 0.141 | 0.544 | 0.382 | <LOQ |
| Pepper (black) | 0.141 | 0.544 | 0.128 | <LOQ |
| Pepper (white) | 0.141 | 0.544 | 0.099 | <LOQ |
| **Meat** | | | | |
| Beef (cooked) | 0.206 | 0.659 | 0.177 | <LOQ |
| Chicken (cooked) | 0.206 | 0.659 | 0.105 | <LOQ |
| Pork (cooked) | 0.206 | 0.659 | 0.085 | <LOQ |
| Turkey (cooked) | 0.206 | 0.659 | 0.125 | <LOQ |
| **Various** | | | | |
| Apple juice | 0.141 | 0.544 | 0.118 | <LOQ |
| Bovine gelatin | 0.141 | 0.544 | 0.141 | <LOQ |
| Bovine serum albumin | 0.141 | 0.544 | 0.133 | <LOQ |
| Cocoa (powder) | 0.141 | 0.544 | 0.128 | <LOQ |
| Coconut | 0.141 | 0.544 | 0.131 | <LOQ |
| Fetal calf serum | 0.134 | 0.484 | 0.088 | <LOQ |
| Fish gelatin | 0.141 | 0.544 | 0.150 | <LOQ |
| Orange juice | 0.141 | 0.544 | 0.121 | <LOQ |
| Porcine gelatin | 0.141 | 0.544 | 0.173 | <LOQ |
| Skim milk (powder) | 0.141 | 0.544 | 0.140 | <LOQ |
| Soya lecithin | 0.141 | 0.544 | 0.220 | <LOQ |
| Wheat starch | 0.141 | 0.544 | 0.169 | <LOQ |
| Whole egg (powder) | 0.141 | 0.544 | 0.150 | <LOQ |

* Results are derived from different runs, i.e. slightly different absorbance values of standards were obtained

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Due to the high potential of cross reactivity to nuts, the reactivity of the ELISA to almonds and hazelnuts was examined in detail (table 6).

**Table 6:** Determination of cross reactivity to roasted almonds and hazelnuts.

| Sample description | OD Std 1 | OD Std 2 | OD Sample | Result (mg/kg) |
|---|---|---|---|---|
| **Almond** | | | | |
| Untreated | 0.185 | 0.680 | 0.131 | <LOQ |
| Roasted 10 min 130 °C (266 °F) | 0.185 | 0.680 | 0.126 | <LOQ |
| Roasted 10 min 150 °C (302 °F) | 0.185 | 0.680 | 0.147 | <LOQ |
| Roasted 10 min 180 °C (356 °F) | 0.185 | 0.680 | 0.129 | <LOQ |
| Roasted 10 min 200 °C (392 °F) | 0.185 | 0.680 | 0.123 | <LOQ |
| **Hazelnut** | | | | |
| Untreated | 0.185 | 0.680 | 0.140 | <LOQ |
| Roasted 10 min 130 °C (266 °F) | **0.185** | 0.680 | 0.125 | <LOQ |
| Roasted 10 min 150 °C (302 °F) | 0.185 | 0.680 | 0.158 | <LOQ |
| Roasted 10 min 180 °C (356 °F) | **0.185** | 0.680 | 0.173 | <LOQ |
| Roasted 10 min 200 °C (392 °F) | 0.185 | 0.680 | 0.144 | <LOQ |

## Recovery experiments

To measure the accuracy, zero samples have been spiked with an extract as described in appendix 2.

**Table 7:** Different spike levels for various food samples.

| Declaration | Final dilution | Concentration calculated (mg/kg) | Concentration measured (mg/kg) | Recovery (%) |
|---|---|---|---|---|
| Chocolate, peanut free | 20 | 0 | < LOQ | |
| Peanut oil, peanut free | 20 | 0 | < LOQ | |
| Butter cookie, peanut free | 20 | 0 | < LOQ | |
| **Fapas rounds peanut in chocolate** | | | | |
| FAPAS 2741 B | 20 | 14.5 | 16.5 | 113.8 |
| FAPAS 2752 B | 20 | 20.2 | 23.4 | 115.8 |
| FAPAS 2766 B | 20 | 22.2 | 19.7 | 88.7 |
| FAPAS 2776 B | 40 | 40.1 | 39.3 | 98.0 |
| Pilot plant produced cookies with defined amounts of peanut | | | | |
| Cookies | 20 | 0 | < LOQ | |
| Cookies | 20 | 5 | 5.8 | 116.0 |
| Cookies | 20 | 10 | 11.2 | 112.0 |
| Cookies | 40 | 20 | 21.7 | 108.5 |
| **Pilot plant produced chocolate with defined amounts of peanut (Par. 35 collab study samples)** | | | | |
| Chocolate, peanut free | 20 | 0 | < LOQ | |
| Dark chocolate | 20 | 10 | 12.9 | 129.0 |
| Dark chocolate | 80 | 40 | 48.7 | 121.8 |
| Dark chocolate | 160 | 80 | 79.5 | 99.3 |
| Milk chocolate | 20 | 5 | 5.9 | 118.0 |
| Milk chocolate | 20 | 10 | 11.7 | 116.6 |
| Milk chocolate | 80 | 20 | 21.3 | 106.4 |
| Milk chocolate | 20 | 40 | 46.6 | 116.4 |
| Candy | 20 | 10 | 13.2 | 132.2 |

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**Table 8:** Different spike levels for various food samples.

| Matrix | Spike (mg/kg) | Concentration found (mg/kg) | Recovery (%) |
|---|---|---|---|
| Cereals | 4.5 | 5.4 | 120.7 |
| | 5.0 | 5.3 | 105.3 |
| | 6.0 | 6.2 | 102.9 |
| | 11.1 | 10.9 | 97.7 |
| Chocolate | 5.0 | 4.4 | 88.0 |
| | 5.4 | 4.6 | 85.2 |
| | 5.5 | 5.4 | 98.2 |
| | 6.5 | 6.2 | 95.8 |
| | 10.0 | 9.5 | 94.5 |
| Cookies | 4.9 | 4.8 | 98.0 |
| | 5.0 | 4.6 | 92.0 |
| | 6.2 | 5.6 | 89.8 |
| Ice cream | 5.8 | 6.2 | 107.3 |
| | 6.0 | 6.1 | 99.2 |
| | 9.1 | 9.0 | 98.9 |
| | 10.3 | 9.8 | 95.1 |
| | 15.5 | 13.0 | 83.5 |
| Overall mean | | | 97.2 |

**Spiked processed matrices**

Food processing such as roasting can alter protein structures and therefore influence antibody binding which, as a consequence, can lead to reduced recoveries.

For untreated peanuts, an overestimation was measured of approx. 350 %. It should be noted that untreated peanuts are very rarely used in commercial foods. The overestimatin of untreated peanuts is due to the standardization to the NIST peanut butter 2387, which contains roasted peanuts. The degree of roasting of the NIST peanut butter is also the range of roasting used for of the vast majority of peanuts in commercial foods. Roasting at higher temperatures reduces the recovery significantly. Please note that these high levels of roasting are rarely used as the peanut color changes to dark brown and the taste strongly deteriorates.

**Table 9:** Recovery (%) of peanuts at different degrees of roasting.

| Sample | Peanut concentration (mg/kg) | Measured concentration (mg/kg peanut) | Recovery (%) |
|---|---|---|---|
| Peanut untreated | 10 | 34.7 | 347 |
| Peanut, roasted 10 min at 130 °C (266 °F) | 10 | 10.5 | 105 |
| Peanut, roasted 10 min at 150 °C (302 °F) | 10 | 10.9 | 109 |
| Peanut, roasted 10 min at 180 °C (356 °F) | 10 | < LOQ | < 25 (measured below LOQ) |
| Peanut, roasted 10 min at 200 °C (392 °F) | 10 | < LOQ | < 25 (measured below LOQ) |

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### Spiked spices

Spices contain large amounts of polyphenols, inhibitors and disturbing substances which might have an impact on any analytical method. Therefore, a wide panel of spices as well as cocoa was spiked according to appendix 2 and analyzed for recovery.

**Table 10:** Recovery (%) of spiked spices and cocoa using extraction with skim milk powder.

| Sample | Expected concentration (mg/kg) | Concentration found (mg/kg) | Recovery (%) |
|---|---|---|---|
| Anise seed | 10 | 9.5 | 95 |
| Basil | 10 | < LOQ | < 25 |
| Caraway | 10 | 6.0 | 60 |
| Cinnamon | 10 | 7.6 | 76 |
| Cloves | 10 | < LOQ | < 25 |
| Cocoa | 10 | 5.6 | 56 |
| Cumin | 10 | 8.3 | 83 |
| Curcuma | 10 | 8.7 | 87 |
| Fennel seed | 10 | 6.3 | 63 |
| Garlic | 10 | 9.6 | 96 |
| Ginger | 10 | 9.1 | 91 |
| Marjoram | 10 | < LOQ | < 25 |
| Mustard seeds, black-brown | 10 | 9.1 | 91 |
| Mustard seeds, yellow | 10 | 6.5 | 65 |
| Nutmeg | 10 | 10.5 | 105 |
| Paprika powder, hot | 10 | 7.1 | 71 |
| Pepper, black | 10 | 5.5 | 55 |

For some spices in table 10, recoveries below 60 % were measured due to the high polyphenol content and the mentioned disturbing effects. However, spices are usually only a minor component in final foods. Therefore, the spices were tested as samples containing 90 % cookie and 10 % of the respective spice. These samples were spiked again and recoveries are shown in the following table.

**Table 11:** Spices tested at 10 % level.

| Sample composition | Expected concentration (mg/kg) | Concentration found (mg/kg) | Recovery (%) |
|---|---|---|---|
| Cookie + 10 % basil | 10 | 7.4 | 74 |
| Cookie + 10 % cloves | 10 | 7.8 | 78 |
| Cookie + 10 % marjoram | 10 | 4.9 | 49 |
| Cookie + 10 % pepper | 10 | 7.3 | 73 |

Also cocao was tested below 60 % in table10. Since cocoa is mainly found in chocolate, several commercial chocolates with varying cocoa content were spiked and tested for recovery.

**Table 12:** Chocolates spiked with peanut.

| Sample composition | Cocoa content (%) | Expected concentration (mg/kg) | Concentration found (mg/kg) | Recovery (%) |
|---|---|---|---|---|
| Chocolate 1 | 35 | 10 | 9.4 | 94 |
| Chocolate 2 | 50 | 10 | 9.4 | 94 |
| Chocolate 3 | 70 | 10 | 9.6 | 96 |

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## Samples from the market

Various samples have been collected from the market and analyzed. Presence of the allergen referring to the manufacturer's declaration was obtained for some samples. Please note that high sample dilutions lead to an increased measurement uncertainty. Furthermore, different processing conditions can have an impact on recovery.

**Table 13:** Measurement of samples from the market.

| Sample description | Declaration by the manufacturer | Optical density | Concentration found (mg/kg) | Recovery (%) |
|---|---|---|---|---|
| Std 1 | | 0.209 | | |
| Std 2 | | 0.675 | | |
| **Samples containing peanut (additional dilution factor of the extract)** | | | | |
| Cereal bar 1 (1:31000) | 31 % peanuts | 2.287 | 448900 | 145 |
| Cereals (1:7000) | 7 % peanuts | 2.149 | 92384 | 132 |
| Chocolate cream (1:25000) | 25 % peanuts | 1.198 | 109710 | 44 |
| Cookie 1 (1:12000) | 12 % peanuts | 1.483 | 80106 | 67 |
| Cookie 2 (1:21000) | 21 % peanuts | 1.855 | 221634 | 106 |
| Cookie 3 (1:19000) | 19 % peanuts | 1.371 | 105644 | 56 |
| **Samples with possible traces of peanuts or nuts in general** | | | | |
| Cereals 2 | May contain traces of peanut | 0.683 | 2.5 | |
| Cookie 4 | May contain traces of peanut | 1.112 | 4.0 | |
| Cookie 5 | May contain traces of peanut | 0.236 | < LOQ | |
| Cookie 6 (1:5) | May contain traces of peanut | 4.085 | > 100 | |
| Chocolate 1 | May contain traces of peanut | 0.232 | < LOQ | |
| Cereal bar 2 | May contain traces of nuts | 1.386 | 5.7 | |
| Chocolate 1 | May contain traces of peanut | 0.234 | < LOQ | |
| Ice cream 1 | May contain traces of peanut | 0.212 | < LOQ | |
| Muesli 1 | May contain traces of peanut | 1.120 | 4.0 | |
| **Samples with no declaration of possibly contained peanuts** | | | | |
| Chocolate 2 | | 0.238 | < LOQ | |
| Chocolate 3 | | 4.206 | > 20 | |
| Chocolate 4 | | 0.184 | < LOQ | |
| Cookies 7 | | 0.446 | < LOQ | |
| Ice cream 2 | | 0.199 | < LOQ | |
| Ice cream 3 | | 0.180 | < LOQ | |
| Muesli 2 | | 0.217 | < LOQ | |
| Rice crispies | | 0.183 | < LOQ | |

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## Additional information

Change of the concentrate of the Allergen extraction buffer (October 2012)

The old kit version contained a 20fold concentrate of the Allergen extraction buffer (AEB). Since October 2012 a new version contains a 10fold concentrate. Both buffers have to be diluted to the same final concentration of the buffer (1fold). The buffer composition was not changed and as, after the correct dilution, the buffer is the same in both cases, the same results were expected.

Before changing the format of the buffer concentrate a re-validation has been made under the supervision of AOAC Research Institute according to an agreed protocol. Negative matrices and spiked samples were measured to proof the assumption. Peanut free milk chocolate and breakfast cereals were extracted 30 times and all extracts were tested well below the LOQ (data not shown). Additionally, the matrices were also spiked with 5 mg/kg NIST peanut butter and extracted 30 times. All samples were detected positive.

**Table 14:** Results of two matrices (chocolate, cereals) spiked with a target value of 5 mg/kg peanut using the 10fold AEB for 30 extractions

| | Milk chocolate spiked with 5 mg/kg peanut | Breakfast cereals spiked with 5 mg/kg peanut |
|---|---|---|
| Mean value (mg/kg peanut) | 5.4 | 4.7 |
| Recovery (%) | 108 | 94 |
| Standard deviation | 0.37 | 0.37 |
| CV (%) | 6.9 | 7.9 |

**Ruggedness** of the test was investigated with small modifications to the regular test performance with regard to temperature (18 - 30 °C), incubation time, and volumes. The modifications applied did not significantly affect the test results (data not shown).

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## Results of collaborative studies

In addition, RIDASCREEN®FAST Peanut was validated with cereals, cookies, ice cream and chocolate in an independent study by three independent US laboratories under the supervision of AOAC Research Institute. All samples were prepared and distributed by FDA/CFSAN (College Park, MD). The FDA laboratory prepared 60 blank samples and 60 samples spiked at a level of 5 mg/kg peanut of each matrix and randomized the samples. Randomized sets of 20 blank and 20 spiked samples of each matrix were sent to each of the three independent laboratories. Samples have been prepared and analyzed according to a protocol of the AOAC Research Institute.

In table 15 a summary of the test results generated by the three independent laboratories is given. All of the spiked samples (240/240) were found positive (sensitivity = 100 %). Two out of 240 blank samples were measured positive (> 2.5 mg/kg peanut) corresponding to a specificity of 99.2 % (238/240).The mean value of all 240 spiked samples was 5.2 mg/kg with a recovery of 104 %.

**Table 15:** Summary of the test results analyzed by three independent laboratories

| | Found | | % Agreement |
|---|---|---|---|
| Target value | Positive | Negative | |
| Positive | 240 | 0 | 100 |
| Negative | 2 | 238 | 99.2 |

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## 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 described ELISA a sensitive and reliable ELISA is available which allows a quantitative and fast determination of the allergen or parts of it in food including all tested matrices.

## Limitations

Samples tested negative still could contain an allergen contamination below the limit of detection of the assay, or they might contain other allergen components like lipids for example.

Due to the multitude of food types, matrix effects cannot be excluded. In processed food (e.g. heat treatment, dehydration, etc.), proteins may be altered or fragmented, this may have an impact on the recovery/cross reactivity.

For evaluation of the cross reactivity only one exemplary sample was analyzed, other samples may show a different result. Cross reactivities of the used antibodies have been determined for the pure food (e.g. corn flour). In a composed/processed food (e.g. corn bread) cross reactivities might be different. Interfering substances (e.g. polyphenols) can be detected by spike experiments.

For spiking, exemplary matrices/foods have been used.

The protein content and the protein composition may vary considerably between different peanut species. Therefore, different varieties may produce different results, since exemplary varieties were used for calibration.

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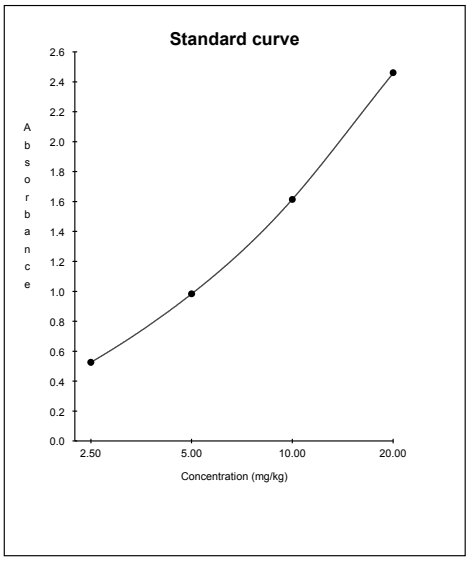
## Appendix 1 – QA Certificate

# Quality Assurance Certificate

**RIDASCREEN®FAST Peanut**

| | |
|---|---|
| REF Art. No. | R6202 |
| LOT Lot | 14515 |
| Expiry | 2017-05 |




Standards

| Std. Conc.(mg/kg) CV(%) | n mean |
|---|---|
| Std1 0.00 8.1 | 4 0.083 |
| Std2 2.50 4.8 | 4 0.526 |
| Std3 5.00 8.6 | 4 0.984 |
| Std4 10.00 6.4 | 4 1.614 |
| Std5 20.00 2.3 | 4 2.461 |

| | Lot. No. | Expiry |
|---|---|---|
| Microtiter plate | 15425 | 2017-06 |
| Allergen extraction buffer | 13435 | 2018-09 |
| Standards | 12505 | 2017-05 |
| Wash buffer | 14495 | 2017-11 |
| Conjugate | 12505 | 2017-05 |

| | Lot. No. | Expiry |
|---|---|---|
| Red Chromogen Pro | 14234 | 2018-04 |
| Stop solution | 11255 | 2020-05 |

IFU (and other accompanying documents where applicable)

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

**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 section 8. Indication of instability or deterioration of reagents.

**R-Biopharm AG,** Quality Assurance Department

**Date:** 2015-12-17

Quality Management System certified ISO 9001 ISO 13485 DQS, Germany

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

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# RIDASCREEN®FAST Peanut

Art. No. R6202

## Appendix 2 – Spike instructions

### 1. Spike with NIST RM® 2387 peanut butter

The National Institute of Standards and Technology (NIST) in the USA offers a Standard Reference Material® 2387 Peanut butter which has a concentration of 90 % peanut.

- Dissolve 22 mg of NIST peanut butter in 20 ml Allergen extraction buffer and shake for 2 hours upside down or by a rotator (concentration: 1 mg/ml peanut).
- Dilute 1:10 with Allergen Extraction buffer (e.g. 500 µl of stock solution + 4500 ml of Allergen extraction buffer) and vortex thoroughly (concentration: 100 µg/ml peanut).
- Add 100 µl of this solution to 1 g of sample to obtain a 10 mg/kg spike.
- Extract the sample as described in the instructions for use of RIDASCREEN®FAST Peanut.

### 2. Spike with American Peanut Butter

American Peanut Butter from the supermarket (e.g. Barney's Best Peanut Butter) contains approx. 90 % of roasted peanut.

- Dissolve 22 mg of peanut butter in 20 ml Allergen Extraction buffer and shake for 2 hours upside down or by a rotator (concentration: 1 mg/ml peanut).
- Dilute 1:10 with Allergen extraction buffer (e.g. 500 µl of stock solution + 4500 ml of Allergen Extraction buffer) and vortex thoroughly (concentration: 100 µg/ml peanut).
- Add 100 µl of this solution to 1 g of sample to obtain a 10 mg/kg spike.
- Extract the sample as described in the instructions for use of RIDASCREEN®FAST Peanut.

# RIDASCREEN®FAST Peanut

Art. No. R6202

## Appendix 3 – Equipment

**Pipettes:**
- 10 - 100 µl Eppendorf Research Plus, Eppendorf
- 20 - 200 µl Eppendorf Research Plus, Eppendorf
- 100 - 1000 µl Eppendorf Research Plus, Eppendorf
- Multichannel: 30 - 300 µl Eppendorf Research Plus, Eppendorf

**Tips:**
- epT.I.P.S.® Standard/Bulk 2 - 200 µl, Eppendorf
- epT.I.P.S.® Standard 50 - 1000 µl, Eppendorf

**Multistepper:**
- Multipette® Xstream Eppendorf Research Plus, Eppendorf

**Multistepper tips:**
- 2.5; 5 and 50 ml Combitips Advanced®, Eppendorf

**Serological pipette:**
- 5 ml and 25 ml CELLSTAR® Serological Pipette, Greiner Bio-One

**Pre-plate:**
- Mikrotiter Assembly Breakable Strip 1x8, Thermo Scientific
- Low binding from Greiner Bio-One, Cat.-No. 655101

**Photometer:**
- Tecan Sunrise, Tecan

**Wash:**
- Dispensette®, Brand
- Brand Tech® 8-channel Manifold, Brand

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02/2016