R-Biopharm AG

# RIDA®QUICK Soya

## Art. No. R7103

**Validation Report**


R-Biopharm – for the safety of your analysis.

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## General

Soy 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 a 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 production of oils and fats.

Soy protein is widely used in food technology to improve functional properties such as foaming, gelling, or emulsification during processing. The increasing use of soy as a food may increase the prevalence of soy allergies. Several soy proteins can cause allergic reactions. The symptoms range from mild rashes like dermatitis, diarrhea and nausea, to life threatening systemic anaphylaxis. Dose-response experiments showed that different amounts of soy protein trigger a food allergy to soy. Objective allergic reactions may appear at about 5 mg soy protein. Often, soy allergic people react also to other leguminosae like beans or peanut and some react to cow's milk or house dust mite, too. Different soy materials with varying protein concentrations are used in the food industry as shown in table 1. The proteins comprise approx. 10 % albumins and 90 % seed storage globulins. The major proteins are glycinin (Gly m 6) with 40 %, β-conglycinin (Gly m 5) with 35 % and 6 % Kunitz soybean trypsin inhibitor. To date, 16 IgE binding soya proteins have been described but not all of them are well characterized.

Due to the multitude of food types, matrix effects cannot be excluded. In processed food, proteins may be altered or fragmented, this may have an impact on the recovery.

In order to ensure a high analytical performance:

- Use also soy free and soy containing (spiked) samples as test controls (e.g. Art. No. R7132)
- Carry out spiking experiments (see appendix 1)
- Confirm results with PCR (e.g. SureFood® Allergen Soya, Art.No. S3401, S3101, S3201)

**Table 1:** Protein content of various soya products

| Soy material Protein content in | % |
|---|---|
| Soybean, green raw (incl. water) | 13 |
| Soybeans, mature seeds, raw | 35 |
| Soy flour, defatted | 47 |
| Soy flour, full fat | 35 |
| Soy protein concentrate | 70 |
| Soy protein isolate | 90 |

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## Sample Preparation

**Extraction**

RIDA®QUICK Soya (R7103) can be directly used after a swab test for surfaces with a short sample extraction (swab, process, dip, read). For the analysis of food samples, an extraction as described in an Application Note for the RIDA®QUICK Soya is necessary.

Therefore, the sample should be extracted with the Extraction buffer of RIDA®QUICK Soya. Only matrices described in that Validation report are tested. Please further validate undescribed food matrices.

## Calibration Curve

The RIDA®QUICK Soya is a qualitative test for soy contamination and does not contain a calibration curve. Results are read visually. Generally, the higher the analyte level in the sample, the stronger the red colour of the test line will be.

## Specificity

The antibodies utilized for RIDA®QUICK Soya dip stick detect especially highly processed soy proteins. In food samples the soy proteins are present in their native form or they may be denatured. Flour, isolates or concentrates, can be used. To ensure a complete detection of soy proteins by the antibody all samples have to be cooked for 5 min for swab samples and 10 min for food samples at 100 °C to convert potentially present native proteins into their denatured form. RIDA®QUICK Soya detects predominantly heated glycinin. (The cross reactivity to heated pure glycinin is approx. 408 %, to heated pure β-conglycinin is approx. 7.3 % and to heated trypsin inhibitor approx. 0.46 %). In fact, the assay overestimates glycinin and underestimates the remaining soy proteins.
The test system is able to detect specifically denaturized soy proteins from raw soybeans, flour, protein concentrates and various products made from soybeans. ‘Soy protein isolates’ are chemically treated products, the recovery of different isolates may vary from 5 - 100 % depending on the preparation. Hydrolyzed soy products may contain only small protein fragments which cannot be fully detected by the dip stick.

## Sensitivity

**Swab Testing**

The sensitivity of the dip stick was analyzed after swabbing a known concentration from a surface of 100 cm².
A surface of 100 cm² was contaminated with concentrations of 0, 2.5, 5, 10 and 20 mg/kg soy protein.
The dip sticks were prepared according to the instruction for use. The test was performed in five replicates and five independent observers read the result of the dip stick. The test was performed twice. Results are shown in Table 2 and Figure 1.

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**Table 2:** Swabbing of different concentrations of soy on surfaces. Concentrations 0, 2.5, 5, 10 and 20 mg/kg soy protein were used to contaminate a surface of 100 cm², swabbed and processed according to the instruction for use. This was repeated five times for each concentration and analyzed by five different operators. The test was performed twice.

| Concentration mg/kg/100 cm² | Replicate | Operator 1 | Operator2 | Operator 3 | Operator 4 | Operator 5 |
|---|---|---|---|---|---|---|
| 0 | 1 | – | – | – | – | – |
| | | – | – | – | – | – |
| | 2 | – | – | – | – | – |
| | | – | – | – | – | – |
| | 3 | – | – | – | – | – |
| | | – | – | – | – | – |
| | 4 | – | – | – | – | – |
| | | – | – | – | – | – |
| | 5 | – | – | – | – | – |
| | | – | – | – | – | – |
| 2.5 | 1 | + | + | – | + | + |
| | | + | + | + | + | + |
| | 2 | – | – | – | + | – |
| | | + | + | – | + | + |
| | 3 | + | – | – | + | + |
| | | + | – | – | + | + |
| | 4 | – | + | – | + | + |
| | | – | – | – | + | + |
| | 5 | – | – | – | – | + |
| | | – | + | – | + | + |
| 5 | 1 | – | + | – | + | + |
| | | + | + | + | + | + |
| | 2 | – | + | – | + | + |
| | | + | + | + | + | + |
| | 3 | – | + | + | + | + |
| | | – | + | + | + | + |
| | 4 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 5 | + | + | – | + | + |
| | | + | + | – | + | + |
| 10 | 1 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 2 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 3 | + | + | – | + | + |
| | | + | + | – | + | + |
| | 4 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 5 | + | + | + | + | + |
| | | + | + | + | + | + |
| 20 | 1 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 2 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 3 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 4 | + | + | + | + | + |
| | | + | + | + | + | + |
| | 5 | + | + | + | + | + |
| | | + | + | + | + | + |

"-" negative, "+" postive

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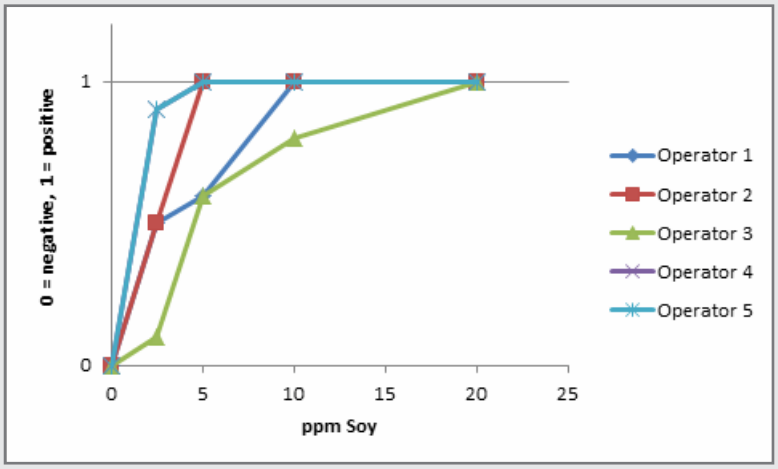


**Figure 1:** Swabbing of different concentrations of soy on surfaces. Concentrations 0, 2.5, 5, 10 and 20 mg/kg soy protein were used to contaminate a surface of 100 cm², swabbed and processed according to the instruction for use. This was repeated five times for each concentration and analyzed by five different operators.
The test was performed twice.

## Cross Reactivity

Approximately 100 compounds were evaluated with the RIDASCREEN®FAST Soya, which uses the same antibody as the RIDA®QUICK Soya. Therefore, it can be supposed that cross reactivity is the same for both systems. For evaluation of the cross reactivity only one exemplary sample was analyzed, other samples may show a different results, all cross reactivities and exemplary analyzed matrices are described in table 5. A cross reactivity was observed for several foods as shown in table 6 and 7. A slight cross reactivity consists with leguminosae of the tribe Phaseoleae (various species of beans), to leguminosae of the genus Vicia and to dried peas and peanut. There is no cross reaction to lentil, fresh peas, lupine and milk or egg proteins. Soy lecithin should not show cross reactivity but sometimes it results in positive detection, in this case soy lecithin is often contaminated with soy proteins.

## Recovery Experiments

### Spiked Processed Matrices

Bread and meat were spiked with a known amount of soy flour prior baking or cooking. Results are shown in table 3 and 4.

**Table 3:** Two different breads were spiked with a known amount of soy prior baking and extracted with the Extraction buffer of RIDA®QUICK Soya three times independently and analyzed two times.

| Sample | Signal of extract 1 | | Signal of extract 2 | | Signal of extract 3 | |
|---|---|---|---|---|---|---|
| Bread mix 50 mg/kg | + | + | + | + | + | + |
| Bread mix 5 mg/kg | + | + | + | + | + | + |
| Rye bread 4000 mg/kg | + | + | + | + | + | + |
| Rye bread 40 mg/kg | + | + | + | + | + | + |
| Rye bread 4 mg/kg | + | + | + | + | + | + |

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**Table 4:** Minced meat was spiked with a known amount of soy prior cooking and extracted with the Extraction buffer of RIDA®QUICK Soya three times independently and analyzed two times, non-diluted and diluted with sample buffer to 4 mg/kg.

| Sample and soy concentration in mg/kg | Extract | Signal of undiluted sample | Signal of diluted sample to a concentration of 4 mg/kg |
|---|---|---|---|
| Sample 1 – contaminated | 1 | + | |
| | 2 | + | |
| | 3 | + | |
| Sample 2 – 40 mg/kg | 1 | + | + |
| | 2 | + | + |
| | 3 | + | + |
| Sample 3 – 400 mg/kg | 1 | + | + |
| | 2 | + | + |
| | 3 | + | + |
| Sample 4 - 4.000 mg/kg | 1 | + | + |
| | 2 | + | + |
| | 3 | + | + |

## 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).

Real time stability of the test will regularly be controlled according to the total quality management schedule of the company.

## Conclusion

RIDA®QUICK Soya is a reliable test system for carrying out swabs for hygiene control and food testing. With the RIDASCREEN®, RIDA® and SureFood® product line it is possible to distinguish between soy-free and soy contaminated samples at very low soy levels and especially for highly processed food. Positive screening results should be quantified using ELISA RIDASCREEN®FAST Soya (R7102) or SureFood® Soya (RS3401, S3101 or S3201).

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