# Soja (Soy) ELISA Kit

Enzyme Immunoassay for the quantitative determination of Soja (Soy) in food

Catalog number: ARG80813

Package: 96 wells

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## INTRODUCTION

Soy (Glycine max) belongs to the legumes. With 39% the fraction of proteins in soy beans is very high. Many of these proteins are known for being aller-genic, such as Gly m1, Glycinin, Kunitz-Trypsin-Inhibitor and Gly m4 which is known to be cross reactive to birch pollen allergen Bet v1. For this reason soy represents an important food allergen. For soy allergic persons hidden soy allergens in food are a critical problem. Already very low amounts of soy can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, soy allergic per-sons must strictly avoid the consumption of soy or soy containing food. Partly undeclared addition of soy as additive in many foods is of particular importance. Cross-contaminations, most-ly in consequence of the production pro-cess are representing another problem. The chocolate production pro-cess is a repre-sentative example. For this reason sensitive detection systems for soy residues in food-stuffs are required. Only a few soy proteins are stable to conventional production processes (for example high temperature). For this reason robust indicator proteins are necessary for detection. Soy trypsin inhibitors (STI) are representing such proteins.

## PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody directed against Soy trypsin inhibitors (STI) is bound on the surface of a microtiter plate. Soy containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against STI is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of STI is directly proportional to the color intensity of the test sample.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody-coated microplate | 12 strips x 8-well | 4°C |
| HRP-antibody Conjugate | 15 ml (ready to use) | 4°C |
| Standards A-E<br>(0, 40, 100, 400, 1000 ppb) | 5 X 2 ml | 4°C |
| 10x Extraction and sample dilution buffer | 2 X 120 ml | 4°C |
| 10x Wash Buffer | 60 ml | 4°C |
| TMB substrate | 15 ml | 4°C<br>(Protect from light) |
| STOP solution | 15 ml | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, Extraction Buffer and Sample diluent buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

## SAMPLE COLLECTION & STORAGE INFORMATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Hazelnut proteins adhere very strongly to different surfaces. In certain cases they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to

note the sequence of the extractions.

**The following sample preparation should be applied for solid samples:**

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 20 mL of **pre-diluted** extraction buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°C. To ensure good homogeneity, the samples should be shaken every two minutes.
3. The samples are centrifuged for 10 minutes at 2000 g. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. **100 µL** of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the **pre-diluted extraction and sample dilution buffer** is necessary. The additional dilution has to be considered when calculating the concentration.

**The following sample preparation should be applied for liquid samples:**

**1 mL** of liquid sample is diluted in **19 mL** of pre-diluted **Extraction & Sample Dilution Buffer.** Afterwards the suspension is incubated for 15 min in a preheated water bath at **60°C**. To ensure good homogeneity, the samples should be shaken every two minutes. The process is continued at point 3 of solid sample extraction process.

Note:

1. Do not shake the final extract to prevent from re-suspension.
2. If after centrifugation a third layer at the top appears due to a high fatty matrix, only the middle aqueous phase should be applied to the wells.

## REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X wash buffer into distilled water to yield 1X wash buffer.
- 1X Extraction and Sample diluent buffer: Dilute 10X Extraction and Sample diluent buffer into distilled water to yield 1X.

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards and samples in duplicate into wells.
3. Incubate for 20 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1X wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add **100 µL** of **HRP-Antibody Conjugate** into each well. Incubate for **20 minutes** at **RT**.
6. Aspirate and wash wells as step 4.
7. Add **100 µl** of **TMB mixture** to each well. Incubate for **20 minutes** at **room temperature** in dark.
8. Add **100 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at 450 nm **immediately**.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. If these quality control criteria (The OD (450/620nm) of the highest standard (1000 ppb, $OD_{1000}$) should > 0.8; the standard $OD_{1000}/OD_0$ should ≥ 5; $CV_0$

should <25%; $CV_{40}$, $CV_{100}$, $CV_{400}$, $CV_{1000}$ should <20%) are not met the assay run is invalid and should be repeated.

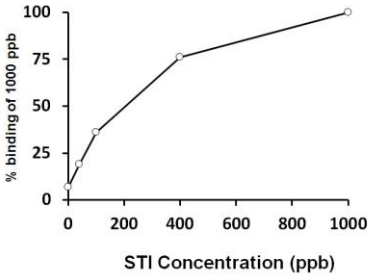
6. The determined amount of STI [ppb] can be used to calculate the amount of the corresponding soy raw product. Therefore the amount of STI has to be multiplied with a conversion factor (F).

The following conversion factors were determined by validation experiments

| | |
|---|---|
| Soy Flour, unroasted | 52 |
| Soy Flour, roasted | 250 |
| Soy Protein Isolate (90%) | 440 |
| Soy Milk | 3700 |
| Textured Soy (Granulate) | 4550 |
| Tofu | 7000 |
| Soy Flour, unroasted | 52 |
| Soy Flour, roasted | 250 |

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

The limit of detection (LOD) of the Soy test is 16 ppb STI.

The limit of quantification (LOQ) of the Soy test is 40 ppb STI.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

### Specificity

For the following foods no cross-reactivity could be detected:

| | | |
|---|---|---|
| Almond | Brazil nut | Ginger, ground |
| Apricot | Buckwheat | Goat's milk |
| Barley | Cabbage, white | Guar gum |
| Bean, white | Caraway | Gum arabic |
| Beef, cooked | Cardamom | Hazelnut |
| Beef, raw | Carob gum | Horseradish |
| Bovine gelatin | Carrot | Isinglass |
| Cashew | Cow's milk | Kidney bean |

| | | |
|---|---|---|
| Cayenne | Crab, cooked | Kiwi |
| Celery | Crab, raw | Lamb |
| Cherry | Cumin | Leek |
| Chervil | Dill | Lentil |
| Chestnut | Duck | Lupin |
| Chia | Egg | Macadamia |
| Chicken | Ewe's milk | Mustard |
| Chickpea | Fennel | Nutmeg |
| Chili | Fish /Cod | Oats |
| Cinnamon | Fish gelatin | Onion |
| Clove | Flaxseed | Paprika |
| Cocoa | Garden cress | Pea |
| Coconut | Garlic, fresh | Peach |
| Corn | Garlic, granulated | Peanut |
| Gliadin | Ginger, fresh | Pecan |
| Pepper, black | Pine seed | Pistachio |
| Plum | Poppy seed | Pork |
| Potato | Prawn, cooked | Prawn, raw |
| Pumpkin seed | Radish | Rapeseed |
| Rice | Rye | Saccharose |
| Sesame | Shrimps | Split pea |
| Sunflower seeds | Thyme | Tomato |
| Turkey | Turmeric | Wheat |
| Walnut | | |

For the following commodities of the table above the results were between 0.5*LOQ and LOQ of the kit. So, it cannot be completely excluded that these ma-trices may provide values above the LOQ in specific cases: Kidney bean

The following cross reactions were determined:
Cross-reactivity for Rapeseed: 0.00001%
Cross-reactivity for Fenugreek: 0.000005%

### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6-8% and the CV value of inter-assay precision was 5-13%

### Recovery

| | |
|---|---|
| Cookies | 106% |
| Cereals | 100% |
| Ice cream | 77% |
| Chocolate | 77% |
| Sausage | 96% |
| Instant soup | 90% |