INSTRUCTION FOR USE

SENSI*Spec* ELISA Soy 96/48 Tests

(Cat. nr. HU0030023 / HU0030047)

GOLD STANDARD DIAGNOSTICS

Enzyme Immunoassay for the Quantitative Determination of Soy in Food

Sensitivity (Soy-Trypsin-Inhibitor)	16 ppb
Recovery	77-106%
Incubation Time	60 min

1. GENERAL INFORMATION

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 allergenic, 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 persons 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, mostly in consequence of the production process are representing another problem. The chocolate production process is a representative example. For this reason sensitive detection systems for soy residues in foodstuffs 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.

The **SENSI*Spec* ELISA Soy** represents a highly sensitive detection system on the base of STI and is particularly capable of the quantification of soy residues in cookies, cereals, ice cream, chocolate, instant soups and sausage.

2. PRINCIPLE OF THE TEST

The **SENSI*Spec* ELISA Soy** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm. The concentration of STI and hence the concentration of soy is directly proportional to the colour intensity of the test sample.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1) Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
2) All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
4) Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
5) Use a separate disposable tip for each specimen to prevent cross-contamination.
6) All specimens and standards should be run at the same time, so that all conditions of testing are the same.
7) Do not mix components from different batches.
8) Do not use reagents after expiration date.
9) Check both precision and accuracy of the laboratory equipment used during the procedure (micropipets, ELISA reader etc.).

4. HEALTH AND SAFETY INSTRUCTIONS

1) Do not smoke or eat or drink or pipet by mouth in the laboratory.
2) Wear disposable gloves whenever handling patient specimens.
3) Avoid contact of substrate and stop solution with skin and mucosa (possible irritation, burn or toxicity hazard). In case of contact, rinse the affected zone with plenty of water.
4) Handling and disposal of chemical products must be done according to good laboratory practices (GLP).

5. REAGENTS

The kit contains reagents for 96/48 determinations. They have to be stored at 2-8°C. Expiry data are found on the labels of the bottles and the outer package.

1) Microtiter plate consisting of 12/6 strips with 8 breakable wells each, coated with anti-STI antibodies.
2) STI Standards (0; 40; 100; 400; 1000 ppb of STI): 5 vials with 2.0 mL each, dyed red, ready-to-use
3) Conjugate (anti-STI-peroxidase): 15/7.5 mL, dyed red, ready-to-use.
4) Substrate Solution (TMB): 15 mL, ready-to-use.
5) Stop Solution (0.5 M H_2SO_4): 15 mL, ready-to-use.
6) Extraction and sample dilution buffer (Tris): 2/1 x 120 mL as 10x concentrate, dyed red. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
7) Washing Solution (PBS + Tween 20): 60 mL as 10x concentrate. Dilute 1+9 with distilled water. Stored at 4°C the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
8) Plastic bag to store unused microtiter strips.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (NOT PROVIDED)

6.1 Instrumentation

- 100 - 1000 µL micropipets
- Volumetric flask
- Analytical balance
- Mortar, mixer
- Water bath
- Centrifuge
- ELISA reader (450 nm)

6.2 Reagents

- Double distilled water

7. SAMPLE PREPARATION

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. 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 and 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.

Attention

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

8. PROCEDURE

The washing solution is supplied as 10x concentrate and has to be **diluted** 1+9 with double distilled water before use.

In any case the **ready-to-use** standards provided should be determined twofold. When a longer series of samples is determined, the standards should be pipetted once before the samples and a second time after the samples. For final interpretation the arithmetic mean is used for calculation.

In consideration of GLP and quality control requirements a duplicate measurement of samples is recommended.

The procedure is according to the following scheme:

1) Prepare samples as described above.
2) Pipet 100 µL **ready-to-use** standards or prepared samples in duplicate into the appropriate wells of the microtiter plate.
3) Incubate for 20 minutes at room temperature.
4) Wash the plate three times as follows: Discard the contents of the wells (dump or aspirate). Pipet 300 µL of diluted washing solution into each well. After the third repetition empty the wells again and remove residual liquid by striking the plate against a paper towel. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbencies.

5) Pipet 100 µL of conjugate (anti-STI-peroxidase) into each well.
6) Incubate for 20 minutes at room temperature.
7) Wash the plate as outlined in 4.
8) Pipet 100 µL of substrate solution into each well.
9) Allow the reaction to develop in the dark (e.g. cupboard or drawer; the chromogen is light-sensitive) for 20 minutes at room temperature.
10) Stop enzyme reaction by adding 100 µL of stop solution (0.5 M H_2SO_4) into each well. The blue colour will turn yellow upon addition.
11) After thorough mixing, measure absorbance at 450 nm (reference wavelength 620 nm), using an ELISA reader. The colour is stable for 30 minutes.

9. CALCULATION OF RESULTS

The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

1) Calculate the average optical density (OD 450 nm) for each set of reference standards or samples.
2) Construct a standard curve by plotting the mean optical density obtained for each reference standard against its concentration in ppb on semi-log graph paper with the optical density on the vertical (y) axis and the concentration on the horizontal (x) axis. Alternatively the evaluation can be carried out by software. In this case the 4-parameter method should be preferred.
3) Using the mean optical density value for each sample, determine the corresponding concentration of STI in ppb from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.

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

Please note: *for calculation of results, it is suggested to use the Excel spreadsheet that is available in the Document Repository section of the Gold Standard Diagnostics web site (www.goldstandarddiagnostics.com).*

10. TYPICAL STANDARD VALUES

The following table contains an example for a typical standard curve. The binding is calculated as percent of the absorption of the 1000 ppb standard. These values are only an example and should not be used instead of the standard curve which has to be measured in every new test.

STI (ppb)	OD-% of 1000 ppb
1000	100
400	76
100	36
40	19
0	7

11. PERFORMANCE

11.1 Sensitivity

The limit of detection (LOD) of the **SENSI*Spec* ELISA Soy test** is 16 ppb STI.

The limit of quantification (LOQ) of the **SENSI*Spec* ELISA Soy test** is 40 ppb STI.

As matrices can have variable influence on the LOD in specific cases, and the range of matrices that was tested is of course limited, the end user if needed may evaluate its own LOD values depending on the matrices to be analyzed.

Alternatively, any results below LOQ should be just reported quantitatively as "< LOQ".

11.2 Cross-reactivity

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

Almond	Crab, cooked	Oats
Apricot	Crab, raw	Onion
Barley	Cumin	Paprika
Bean, white	Dill	Pea
Beef, raw	Duck	Peach
Beef, cooked	Egg	Peanut
Bovine gelatin	Ewe's milk	Pecan
Brazil nut	Fennel	Pepper, black
Buckwheat	Fish/Cod	Pine seed
Cabbage, white	Fish gelatin	Pistachio
Caraway	Flaxseed	Plum
Cardamom	Garden cress	Poppy seed
Carob gum	Garlic, fresh	Pork
Carrot	Garlic, granulated	Potato
Cashew	Ginger, fresh	Prawn cooked
Cayenne	Ginger, ground	Prawn, raw
Celery	Gliadin	Pumpkin seed
Cherry	Goat's milk	Radish
Chervil	Guar gum	Rapeseed
Chestnut	Gum arabic	Rice
Chia	Hazelnut	Rye
Chicken	Horseradish	Saccharose

Chickpea	Isinglass	Sesame
Chili	Kidney bean	Shrimps
Cinnamon	Kiwi	Split pea
Clove	Lamb	Sunflower seed
Cocoa	Leek	Thyme
Coconut	Lentil	Tomato
Corn	Lupin	Turkey
Cow's milk	Macadamia	Turmeric
	Mustard	Walnut
	Nutmeg	Wheat

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 matrices may provide values above the LOQ in specific cases:

Kidney bean

The following cross reactions were determined:

Adzuki Bean	0.00001%
Fenugreek	0.000005%

11.3 Precision

Intra-Assay Precision	6 - 8%
Inter-Assay Precision	5 - 13%

11.4 Linearity

The serial dilution of spiked samples (cookies, cereals, ice cream, chocolate, instant soup and sausage) resulted in a dilution linearity of 81% - 128%.

11.5 Recovery

Mean recovery was determined by spiking samples with different amounts of STI:

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

12. LIABILITY

Gold Standard Diagnostics Budapest shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of the results.

Gold Standard Diagnostics Budapest shall not be liable for the unsafe use of the kit out of the current European safety regulations.