SENSI*Spec* Total Soy Protein ELISA 96/48 Tests

Enzyme Immunoassay for the Quantitative Determination of Soy Protein in Food (Cat.nr. HU0030075 / HU0030076)

Sensitivity	0.2 ppm
Recovery	66 - 113%
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. Soy represents a food which may exist in multiple conditions and processing states. This has to be covered by the analytical method.

For this reason, the **SENSISpec Total Soy Protein ELISA** is based on antibodies raised again hydrolyzed soy protein which can represent the variety of manufacturing processes. As such it is capable of the quantification of soy protein residues in baby food, cookies, cereals, ice cream, chocolate, instant meal and sausage.

2. PRINCIPLE OF THE TEST

The **SENSISpec Total Soy Protein** quantitative test is based on the principle of the enzyme linked immunosorbent assay. An antibody directed against hydrolysed soy protein is bound on the surface of a microtiter plate. Soy protein 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 hydrolysed soy protein 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 soy protein 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.

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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-soy protein antibodies.

2) Soy Protein Standards (0; 2; 6; 18; 36 ppm of soy protein): 5 vials with 2.0 mL each, dyed red, ready-to-use

3) Conjugate (anti-soy protein-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 Buffer (Tris): 2/1 x 120 mL as 10x concentrate. 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) Sample Dilution Buffer A (Tris): 60 mL, dyed red, ready-to-use.

8) Sample Dilution Buffer B (Tris): 60 mL, dyed red, ready-to-use.

9) 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.

10) Plastic bag to store unused microtiter strips.

11) Instruction Manual.

6. ADDITIONAL INSTRUMENTATION AND REAGENTS (not provided)

Instrumentation

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

Reagents

- double distilled water
- Polyvinylpyrrolidone (PVP) for polyphenol containing samples

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 all 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.

3) Afterwards the suspension is incubated for 15 min in a preheated water bath at 100°C. To ensure good homogeneity, the samples should be shaken every two minutes.

4) The samples are centrifuged for 10 minutes at 2000 g or higher. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.

5) The samples are diluted 1:5 in **ready-to-use** sample dilution buffer B for milk products and sausage or in **ready-to-use** sample dilution buffer A for all other samples (e.g. 100 µL prepared sample + 400 µL diluent).

6) 100 µL of prepared and diluted sample are applied per well. If the results of a sample are out of the measuring range, further dilution with the **ready-to-use** dilutent is necessary. The additional dilution has to be considered when calculating the concentration.

The following sample preparation should be applied for liquid samples:

1) 1 mL of liquid sample is diluted in 19 mL of **pre-diluted** extraction buffer. The process is continued at point 3 of solid sample extraction process.

The following variation should be applied for polyphenol containing samples like chocolate:

Dilute 1 g of Polyvinylpyrrolidone (PVP) in 100 mL of **pre-diluted** extraction buffer. Apply the buffer as extraction buffer in the sample preparations stated above

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-soy protein-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 ppm 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 soy protein in ppm 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 soy protein [ppm] can be used to calculate the amount of the corresponding soy raw product. Therefore, the amount of soy protein has to be multiplied with a conversion factor (F).

The following conversion factors were determined by validation experiments:

Soy Flour, unroasted (53% protein)	1.5
Soy Flour, roasted (40% protein)	2.2
Soy Protein Isolate (90% protein)	1.1
Soy Milk (3% protein)	28
Textured Soy (45% protein)	2.3
Tofu (14% protein)	45

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 36 ppm 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.

Soy Protein (ppm)	OD-% of 36 ppm
36	100
18	58
6	26
2	11
0	4

11. PERFORMANCE

11.1. Sensitivity

The limit of detection (LOD) of the **SENSISpec Total Soy Protein test** is 0.2 ppm soy protein.

Validation experiments with common matrices resulted in the following mean LODs [ppm].

Cookies	0.4
Cornflakes	0.4
Ice-cream	0.2
Instant meal	0.5
Sausage	0.4
Baby food	0.5
Cheese	0.3

The limit of quantification (LOQ) of the **Immunolab Total Soy Protein test** is 2 ppm soy protein.

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

NOTE: some legumes may give underestimated values with the kit. Please consult with us when analysing this kind of samples.

11.2. Cross-reactivity

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

Adzuki bean	Crab, cooked	Paprika
Almond	Crab, raw	Pea
Amaranth	Cress	Peanut
Barley	Cumin	Pecan
Bean. white	Duck	Pepper
Beef	Egg	Pine seed
Bovine gelatin	Ewe's milk	Pistachio
Brazil nut	Fenugreek	Plum
Buckwheat	Fish gelatin	Poppy seed
Caraway	Flaxseed	Pork
Carob gum	Gliadin	Potato
Carrot	Goat's milk	Pumpkin seed
Cashew	Guar gum	Quinoa
Cayenne	Hazelnut	Rape seed
Celery	Isinglass	Rice
Cherry	Kidney bean	Rye
Chervil	Kiwi	Saccharose
Chestnut	Lamb	Sesame
Chia	Lentil	Shrimp, cooked
Chicken	Lupin	Shrimp, raw
Chickpea	Macadamia	Spelt
Chili	Millet	Sunflower seeds
Cocoa	Mustard	Tomato
Coconut	Nutmeg	Turkey
Cod	Oats	Walnut
Corn	Onion	Wheat
Cow's milk		

11.3. Precision

Intra-Assay Precision	8.0%
Inter-Assay Precision	6.6%

11.4. Linearity

Linearity

The serial dilution of spiked samples (cookies, cereals, ice cream, chocolate, instant meal and sausage) resulted in a mean dilution linearity of 101%.

11.5. Recovery

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

Cookies	103%
Cereals	92%
Ice cream	77%
Chocolate	79%
Instant meal	94%
Sausage	82%
Baby food	77%
Cheese	74%