INSTRUCTION FOR USE

SENSI*Strip* Total Soy Protein PowerLine 20/5 Tests

(Cat. nr. HU0030135, HU0030146)

Lateral-flow Device for the Determination of Total Soy Protein in Food and as Cleaning Control Monitoring

Sensitivity for food matrix	2.5 ppm
Sensitivity for swabbing	0.007 µg/cm^2
Sensitivity for rinse water	0.33 mg/L

1. GENERAL INFORMATION

Soy (Glycine max) belongs to the legumes. With 39% the fraction of proteins in soybeans 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.

The **SENSI*Strip* Total Soy Protein PowerLine Lateral Flow Device** represents a sensitive detection system based on a monoclonal antibody and is particularly capable to detect soy protein residues in food matrices, rinse water and swabs. Due to an introduced hook line, false negative interpretation of highly contaminated samples (hook effect) can be excluded.

2. PRINCIPLE OF THE TEST

The **SENSI*Strip* Total Soy Protein PowerLine** test is based on the principle of immunoassay. Soy protein containing sample is given into an incubation vial and a test strip is placed into the sample. The sample migrates along the nitrocellulose membrane by capillary forces. Along its way it releases gold nanoparticles conjugated to anti-soy protein-antibodies. For positive samples a red line is formed when the liquid reaches the test line area. In case of negative samples, no line is formed. After passing the test line the liquid passes a second line, being the hook line. A red line is formed in any cases the liquid passes the hook line except the sample is highly contaminated with analyte. In this case the hook line signal decreases with increasing analyte concentration of the sample. In any case, above the test line and hook line areas a red control line appears, indicating the validity of the test. The test is evaluated after 5 minutes.

3. PRECAUTIONS

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

1) Store the kit at 2-8°C.
2) Do not use the kit after its expiry date.
3) Prior to beginning the assay procedure, bring all samples and reagents to room temperature (20-25°C).
4) Dilution buffer should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
5) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
6) Replace caps in all the reagents and samples immediately after use.
7) Use separate disposable consumables for each transfer of sample to the reaction vial in order to prevent cross-contamination.
8) Do not mix components from different batches.
9) Do not use reagents after expiration date.

NOTE: The swab sampling device included in this kit may be supplied as sterile with a sterility expiration date printed on the device. However, this kit does not require a sterile sampling device, therefore the swab sterility expiration date does not affect the kit expiration date and can be disregarded.

4. KIT CONTENTS

The kit contains components and reagents for 20 tests or 5 tests. They have to be stored at 2-8°C. Expiry data are printed on the labels of the reagent containers and the outer package.

Content	20-strip	5-strip
Test Strips, in tube with desiccant stopper	20 pcs	5 pcs
Incubation vials	20 pcs	5 pcs
Extraction tubes with caps	20 pcs	5 pcs
Extraction buffer, 60 mL, ready-to-use.	1 pcs	1 pcs
Disposable pipettes, 0.3 mL	21 pcs	6 pcs
Disposable pipette, 3 mL	1 pcs	1 pcs
Disposable spatulas	20 pcs	5 pcs
Swab sticks	20 pcs	5 pcs
Evaluation Card	1 pcs	1 pcs
Tubes and vials racks	by kit box	by kit box
IfU	1 pcs	1 pcs
QR-Code for evaluation with RapidScan ST5 lateral flow strip reader	1 pcs	1 pcs

5. EQUIPMENT AND MATERIALS (NOT PROVIDED)

1) *RapidScan ST5* lateral flow reader for quantitative evaluation (optional)

6. SAMPLE PREPARATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, vials etc. have to be **cleaned thoroughly** before and after each sample. Allergen 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 for pattern recognition.

FOOD SAMPLES (STANDARD METHOD)

1) Homogenize sample using appropriate methods depending on its specific nature (e.g. grind, crush, mix).
2) *Solid samples:* Transfer one spatula of sample to an extraction tube. Alternatively, in order to increase precision, weigh out 0.2 g of sample into an extraction tube.
   Liquid samples: Transfer a half spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.2 mL of sample into an extraction.
3) Add 3 mL of ready-to-use extraction buffer to the sample by using the disposable 3 mL pipette.
4) Close extraction tube with cap and shake for 1 minute.
5) Let the solid remains sediment. Depending on nature of the samples this might take 1-2 minutes. Alternatively centrifuge at 2000 g or higher.
6) Remove cap and transfer 0.3 mL of sample supernatant into an incubation vial using a disposable 0.3 mL pipette.

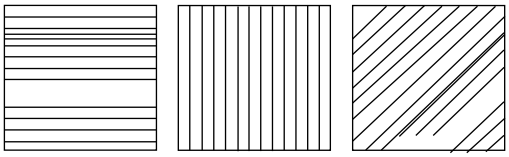
RINSE WATER

1) In case of strong acidic or basic rinse solution adjust the pH of the sample to 7 (+/- 0.5).
2) Transfer 0.3 mL of dilution buffer into a dilution tube using one of the disposable 0.3 mL pipettes.
3) Transfer 0.3 mL of rinse sample into the extraction tube using a second disposable 0.3 mL pipette.
4) Mix the two liquids by applying the same pipet as in step 3.
5) Transfer 0.3 mL of mixture to an incubation vial applying the same pipet as in step 4.

SWABBING SAMPLES

DRY SURFACES

1) Mark out 5x5 cm area or use swab directly on (e.g. uneven) area.
2) Transfer 1 mL of ready-to-use dilution buffer into an extraction tube by using one of the disposable 3 mL pipettes.
3) Moisten a swab by dipping into the tube.
4) Swab marked area by using crosshatch (1. horizontally, 2. vertically, 3. diagonally) technique while rotating the tip.



5) Place swab into the tube and break off the tip.
6) Close extraction tube with cap and shake for 1 minute to release the sample from the swab.
7) Remove cap and transfer 0.3 mL of sample supernatant into an incubation vial using a disposable 0.3 mL pipette.

WET SURFACES

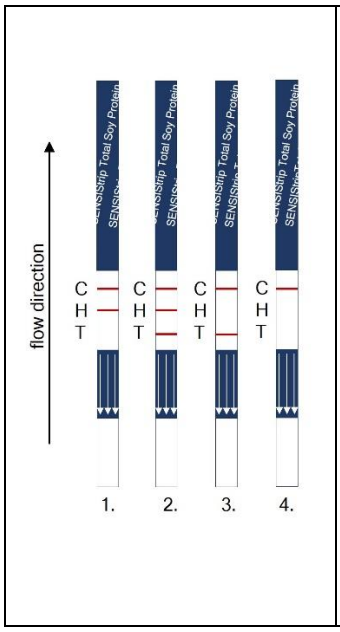
Apply same method as described for dry surfaces without prior need to moisten the swab.

7. ASSAY PROCEDURE

1) Prepare samples as described above.
2) After transfer of sample place one strip into the incubation vial. For proper strip orientation make sure that the arrows on the cover foil point downwards.
3) Incubate for 5 minutes.
4) Remove strip from the vial and evaluate immediately.

8. EVALUATION

SENSI*Strip* PowerLine lateral-flow devices are evaluated according to the following scheme:



1. **Negative:** visible control (C) line, visible hook (H) line, no test (T) line
2. **Positive:** visible control (C) line, hook (H) line and test (T) line
3. **Positive (Hook Effect):** visible control (C) line, visible test (T) line, no hook (H) line
4. **Strongly Positive (Strong Hook Effect:):** visible control (C) line, decreased or no test (T) line, no hook (H) line

In any case the control (C) line is not visible the test has to be treated as invalid.

For a better distinguishing between negative, borderline and positive samples a colour card for evaluation is provided with the kit. The intensity of the test line and hook line has to be compared with the different increments of the colour card. Test line results lower than increment 3 should be treated as negative. Test line results according increment 3 or higher should be treated as positive. The test is adjusted to a hook effect beginning with a concentration between 500-1000 ppm. With further increasing analyte concentration and thus hook effect the test line result also starts to decrease. Hook line results lower than increment 3 should be treated as hook effect. In that case, the sample should be further diluted to prevent false-negative results for highly contaminated samples.

Since the increments of the colour card are ranging up to 10 a semi-quantitative evaluation is also possible. This can be improved by taking into account the results stated in the validation report of the product.

In addition, a quantitative evaluation (2.5-50 ppm) in combination with the *Gold Standard Diagnostics RapidScan ST5* lateral flow reader is possible. The method includes the indication of the hook effect beginning with the above stated concentration. For further information please contact Gold Standard Diagnostics.

9. PERFORMANCE

9.1 Sensitivity

LOD (total soy protein) of the SENSI*Strip* lateral-flow test is 2.5 ppm for food matrix, 0.33 mg/L for rinse water and 0.007 µg/cm^{2}for swab samples applying the procedure above.

Note: Sensitivity may vary depending on matrix and processing of a complex food mixture. For achieving reliable results each matrix should be validated prior to routine testing.

9.2 Cross-reactivity

For the following foods no cross-reactivity could be detected applying the standard extraction method:

Adzuki bean	Cow's milk	Oyster
Almond	Cumin	Paprika
Apricot	Curcuma	Pea
Barley	Dill	Peach
Bean, white	Duck	Peanut
Bovine	Ewe's milk	Pecan
Bovine gelatine	Fennel	Pepper
Brazil nut	Fenu greek	Pine nut
Buckwheat	Flaxseed	Pistachio
Caraway	Garden cress	Poppy seed
Cardamom	Garlic	Pork
Carob bean	Gliadin	Potato
Carrot	Goat's milk	Pumpkin seed
Cashew	Guar gum	Radish
Cayenne	Hazelnut	Rice
Celery	Horseradish	Rye
Cherry	Kidney bean	Sesame
Chestnut	Kiwi	Shrimp
Chia	Lamb	Split peas
Chicken	Leek	Sucrose
Chickpea	Lentil	Sunflower seed
Chili	Macadamia	Thyme
Cinnamon	Milk powder	Tomato
Cocoa	Mustard, yellow	Turkey
Coconut	Nutmeg	Walnut
Cod	Oats	Wheat
Corn	Onion	White cabbage

The following cross-reactions were determined:

Clove	0.006%

In case of testing lupin containing samples please consult Gold Standard Diagnostics.

9.3 High-dose-hook Effect

Since the test includes a hook line a hook effect cannot occur without indication (Hook line results lower than increment 3). The test is adjusted to a hook effect beginning between 500 and 1000 ppm for food samples according to 1.3 – 2.7 mg/cm^2 for swabs and 67 - 133 mg/L for rinse water samples.

10. ADDITIONAL PERFORMANCE DATA

Additional data can be found in the corresponding validation report of the product, which can be inquired at Gold Standard Diagnostics.

11. 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 results.

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