INSTRUCTION FOR USE

SENSI*Strip* Sesame PowerLine 20/5 Tests

(Cat. nr. HU0030270, HU0030208)

Lateral-flow Device for the Determination of Sesame in Food and as Cleaning Control Monitoring

Sensitivity for food matrix	5 ppm
Sensitivity for swabbing	0.013 µg/cm^2
Sensitivity for rinse water	0.67 mg/L

1. GENERAL INFORMATION

Sesame belongs to the family of Pedaliaceae. With about 16-32% the fraction of proteins in sesame seed is very high. Some of these proteins, like the albumins Ses i 1 and Ses i 2 or the globulin Ses i 3 are known for being allergenic. Because of its wide-spread application possibilities, sesame is used in many food preparations.

For sesame-allergic persons hidden sesame allergens in food are a critical problem. Already very low amounts of sesame can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, sesame-allergic per-sons must strictly avoid the consumption of sesame containing food. Cross-contamination, most-ly in consequence of the production pro-cess, is often noticed. This explains why in many cases the existence of sesame residues in food cannot be excluded. For this reason, sensitive detection systems for sesame residues in food-stuffs are required.

The Gold Standard Diagnostics Hazelnut Power-Line Lateral Flow Device represents a sensitive detection system and is particularly capable to detect hazelnut 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 **Gold Standard Diagnostics Sesame PowerLine** test is based on the principle of immunoassay. Sesame 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-sesame 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 labora¬tory practices (GLP) will determine the reliabil¬ity 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 in¬duce foaming.
5. Once the assay has been started, all subse¬quent steps should be completed without interruption and within the recommended time limits.
6. Replace caps in all the reagents and samples immedi¬ately 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.

Gold Standard Diagnostics Budapest Kft.
Fóti út 56 A Ép., Budapest, Hungary
https://www.goldstandarddiagnostics.com/contacts

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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 pc	1 pc
Disposable pipettes, 0.3 mL	21 pcs	6 pcs
Disposable pipette, 3 mL	1 pc	1 pc
Disposable spatulas	20 pcs	5 pcs
Swab sticks	20 pcs	5 pcs
Evaluation Card	1 pc	1 pc
Tubes and vials racks	by kit box	by kit box
QR-Code for evaluation with RapidScan ST5 lateral flow strip reader	1 pc	1 pc
Instruction manual	1 pc	1 pc

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

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.

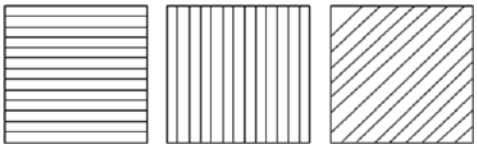
RINSE WATER

1) Add 2% NaCl to sample.
2) In case of strong acidic or basic rinse solution adjust the pH of the sample to 7 (+/- 0.5).
3) Transfer 0.3 mL of dilution buffer into an extraction tube using one of the disposable 0.3 mL pipettes.
4) Transfer 0.3 mL of rinse sample into the extraction tube using a second disposable 0.3 mL pipette.
5) Mix the two liquids by applying the same pipet as in step 4.
6) 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

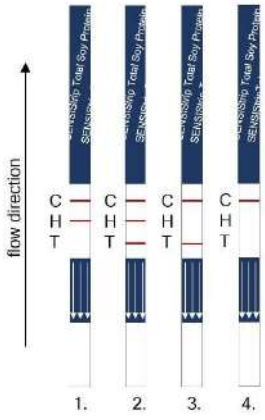
Apply same method as described for dry surfaces without prior need to moisten the swab.

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 160-1000 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 (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 of the SENSI*Strip* lateral-flow test is 5 ppm for food matrix, 0.67 mg/L for rinse water and 0.013 µg/cm2 for swab samples applying the procedure above. The corresponding amounts of hazelnut protein can be calculated by anticipating a protein content of hazelnut of 18%.

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	Curcuma	Peanut
Almond	Dill	Pecan
Apricot	Duck	Pepper
Barley	Egg (1:10)	Pine nut
Beef	Ewe's milk	Pistachio
Bovine gelatine (1:10)	Fennel	Poppy seed
Brazil nut	Fenugreek (1:10)	Pork
Buckwheat	Flaxseed	Potato
Caraway	Garden cress	Psyllium husk (1:10)
Cardamom	Garlic	Pumpkin seed
Carrot	Gliadin (1:10)	Radish
Cashew	Goat's milk	Rice
Cayenne	Guar gum (1:10)	Rye
Celery	Horseradish	Soy flour
Cherry	Kiwi	Soy lecithin
Chestnut	Leek	Soy milk
Chicken	Lentil	Split peas
Chili	Macadamia	Sucrose
Cinnamon	Milk powder	Sunflower seed
Cocoa	Mustard, yellow	Thyme
Coconut	Nutmeg	Tomato
Cod	Oats	Walnut
Corn	Onion	Wheat
Cow's milk	Paprika	White cabbage
Cumin	Peach	

The following cross-reactions were determined:

Bean, white	0.5%
Chia	1%
Carob bean	0.005%
Kidney bean	0.5%

For the following matrices the hook line results were close to a signal of 3 (hook effect), but the sample results <3 (negative):

Chickpea	Lupin	Shrimp
Clove	Oyster	Turkey
Hazelnut	Rapeseed	

Further information can be found in the validation report and/or be inquired at 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 160 and 1000 ppm for food samples according to 0.427 – 2.67 µg/cm² for

swabs and 21.3 – 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.