INSTRUCTION FOR USE

SENSI*Strip* Gluten PowerLine 20/5 Tests

(Cat. nr. HU0030134, HU0030139)

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

Sensitivity for food matrix	4 ppm
Sensitivity for swabbing	1.6 ng/cm^2
Sensitivity for rinse water	0.08 mg/L

1. GENERAL INFORMATION

Gluten is the main part of the protein fraction of cereals and consists of nearly the equal amount of the protein compounds prolamin (gliadin) and glutenin. Because of its special physico-chemical attributes and its low price, gluten is not only contained in cereal products, but also in other food as sausage products and ice cream or in drugs and cosmetics as binder and filler.

For some persons, gluten has a pathological effect (coeliac disease). These people need to have a strict gluten free diet. In the European Union a maximum level of 20 ppm gluten is allowed for products declared as “gluten-free”, and 100 ppm gluten for products declared as “very low gluten” respectively. Sensitive detection systems are required to determine gluten residues in foodstuff.

The **SENSI*Strip* Gluten PowerLine Lateral Flow Device** represents a sensitive detection system based on a monoclonal antibody and is particularly capable to detect gluten 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. Validation experiments have shown that the antibody shows identical behaviour and response against gluten proteins as the R5 antibody.

2. PRINCIPLE OF THE TEST

The **SENSI*Strip* Gluten PowerLine** test is based on the principle of immunoassay. Gluten 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-gluten-antibodies.

1) Extraction tubes with caps, 20 pcs

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
Dilution tubes with caps	20 pcs	5 pcs
Dilution buffer, 60 mL, ready-to-use.	1 pcs	1 pcs
Disposable pipettes, 0.3 mL	21 pcs	6 pcs
Disposable pipettes, 3 mL	3 pcs	3 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) Ethanol (>99 % purity) diluted to 50 % with water (standard extraction method)
2) SENSI*Spec* Ingezim Gluten Extraction Additive, Cat. No. S.30.GLUT07210c/S.30.GLUT07211c (extraction of heat-treated samples)
3) Ethanol (>99 % purity) diluted to 60 % with water (extraction of heat-treated samples)
4) *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.

7. 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 and a half spatula of sample to an extraction tube. Alternatively, in order to increase precision, weigh out 0.3 g of sample into an extraction tube.
   Liquid samples: Transfer one spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.3 mL of sample into an extraction.
3) Add 3 mL of ethanol (50%) to the sample by using one of the disposable 3 mL pipettes.
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 a dilution tube using a disposable 0.3 mL pipette.
7) Add 3 mL of dilution buffer to the sample by using the second of the disposable 3 mL pipettes.
8) Close dilution tube with cap and shake for 1 minute.
9) Remove cap and transfer 0.3 mL of the mixture into an incubation vial using the same 0.3 mL pipette as in step 6.

For milk powder samples it is recommended to do an additional 1:2 dilution with dilution buffer after step 7. Further information can be found in the corresponding validation report of the product, which can be inquired at Gold Standard Diagnostics.

8. HEAT TREATED 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 one spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.2 mL of sample into an extraction tube.
3) Add 500 µL of SENSI*Spec* Ingezim Gluten Extraction Additive to the sample by using one of the disposable 3 mL pipettes.
4) Add 1.5 mL of ethanol (60%) to the sample by using a second disposable 3 mL pipettes.
5) Close extraction tube with cap and shake for 10 seconds.
6) Incubate for 15min at room temperature. To ensure good homogeneity, the samples should be shaken every 3 minutes.
7) Let the solid remains sediment. Depending on nature of the samples this might take 1-2 minutes. Alternatively centrifuge at 2000 g or higher.
8) Remove cap and transfer 0.3 mL of sample supernatant into a dilution tube using a disposable 0.3 mL pipette.
9) Add 3 mL of dilution buffer to the sample by using the second of the disposable 3 mL pipettes.
10) Close dilution tube with cap and shake for 1 minute.
11) Remove cap and transfer 0.3 mL of the mixture into an incubation vial using the same 0.3 mL pipette as in step 8.

8.1 Rinse water

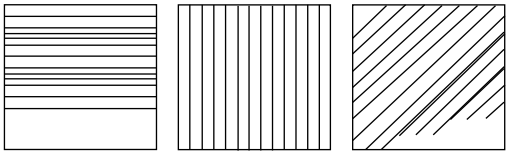
1) In case of strong acidic or basic rinse solution adjust the pH of the sample to 7 (+/- 0.5).

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.

8.2 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

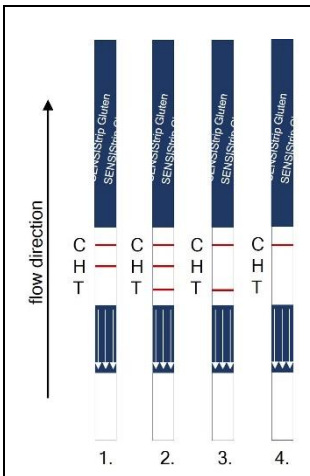
Apply same method as described for dry surfaces without prior need to moisten the swab.

9. ASSAY PROCEDURE

1) Prepare samples as described above.
2) After transfer of sample place one strip into the 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.

10. 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 (4-80 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.

11. PERFORMANCE

All performance data was evaluated based on *Prolamin Working Group Gliadin (PWG-Gliadin).*

11.1 Sensitivity

LOD (gluten) of the SENSI*Strip* lateral-flow test is 4 ppm for food matrix, 0.08 mg/L for rinse water and 1.6 ng/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.

11.2 Cross-reactivity

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

Adzuki bean	Cumin	Pea
Almond	Curcuma	Peach
Amaranth	Dill	Peanut
Anise	Duck	Pecan
Apricot	Ewe's milk	Pepper
Bean, white	Fava bean	Pine nut
Bovine	Fennel	Pistachio
Bovine gelatine	Fenugreek	Poppy seed
Brazil nut	Flaxseed	Pork
Buckwheat	Garden cress	Potato
Caraway	Garlic	Pumpkin seed
Cardamom	Goat's milk	Quinoa
Carob bean	Golden millet	Radish
Carrot	Guar gum	Rice
Cashew	Hazelnut	Sesame
Cayenne	Horseradish	Shrimp
Celery	Kidney bean	Sorghum
Cherry	Kiwi	Soy flour
Chestnut	Lamb	Soy lecithin
Chia	Leek	Soy milk
Chicken	Lentil	Split pea
Chickpea	Lupin	Sucrose
Chili	Macadamia	Sunflower seed
Cinnamon	Milk powder	Tapioca
Clove	Mustard, yellow	Teff
Cocoa	Nutmeg	Thyme
Coconut	Oats	Tomato
Cod	Onion	Turkey
Corn	Oyster	Walnut
Cow's milk	Paprika	White cabbage

The following cross-reaction was determined applying the standard extraction method:

Arrowroot	0.0008%

For the following foods no cross-reactivity could be detected applying the extraction method for heat-treated samples:

Adzuki bean	Curcuma	Peach
Almond	Dill	Peanut
Amaranth	Duck	Pecan
Anise	Ewe's milk	Pepper
Apricot	Fava bean	Pine nut
Bean, white	Fennel	Pistachio
Bovine	Fenugreek	Poppy seed
Bovine gelatine	Flaxseed	Pork
Brazil nut	Garden cress	Potato
Buckwheat	Garlic	Pumpkin seed
Cardamom	Goat's milk	Quinoa
Carob bean	Golden millet	Radish
Carrot	Guar gum	Rice
Cashew	Hazelnut	Sesame
Cayenne	Horseradish	Shrimp
Celery	Kidney bean	Sorghum
Cherry	Kiwi	Soy flour
Chestnut	Lamb	Soy lecithin
Chia	Leek	Soy milk
Chicken	Lentil	Split peas
Chickpea	Lupin	Sucrose
Chili	Milk powder	Sunflower seed
Cinnamon	Mustard, yellow	Tapioca
Clove	Nutmeg	Teff
Cocoa	Oats	Thyme
Coconut	Onion	Tomato
Cod	Oyster	Turkey
Corn	Paprika	Walnut
Cow's milk	Pea	White cabbage

The following cross-reactions were determined applying the extraction method for heat-treated samples:

Arrowroot	0.002%
Caraway	0.002%
Cumin	0.0008%
Macadamia	0.02%

11.3 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 – 2.6 mg/cm^2 for swabs and 66 - 133 mg/L for rinse water samples.

12. ADDITIONAL PERFORMANCE DATA

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

13. 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.