INSTRUCTION FOR USE

SENSI*Strip* Gluten 20/5 Tests

(Cat. nr. HU0030118/HU0030158)

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 such as sausage products and ice cream or in drugs and cosmetics as binder and filler.

For some people, 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 Lateral Flow Device** represents a sensitive detection system based on a monoclonal antibody and is particularly capable of detecting gluten residues in food matrices, rinse water and swabs. Validation experiments have shown that the antibody shows identical behavior and response against gluten proteins as the R5 antibody.

2. PRINCIPLE OF THE TEST

The **SENSI*Strip* Gluten** test is based on the principle of immunoassay. Gluten containing sample is given into a reaction vial containing an activation reagent. After 3 minutes incubation at room temperature a test strip is placed into the reaction vial. The sample migrates along the nitrocellulose membrane by capillary forces. Along its way it releases gold nanoparticles conjugated to anti-gluten-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. In any case, above the test line area a red control line appears, indicating the validity of the test. The test is evaluated after another 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 the 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, there-fore 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
Reaction vials	20 pcs	5 pcs
Extraction tubes with caps	20 pcs	5 pcs
Dilution tubes	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	2 pcs	2 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 (50%)
2) 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.
Chocolate and other products with high polyphenol content tend to show reduced results. To overcome this effect a special extraction additive can be ordered separately (HU0030100).

6.1. Solid samples / Liquid samples

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 the extraction tube with a cap and shake for 1 minute.
5) Let the solid remain sediment. Depending on the 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 vial 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 the dilution tube with a cap and shake for 1 minute.
9) Remove cap and transfer 0.3 mL of the mixture into a reaction vial using the same 0.3 mL pipette as in step 6.

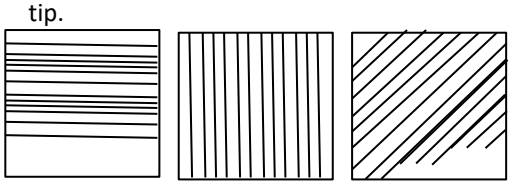
6.2. Rinse water

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 an extraction 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 a reaction vial applying the same pipet as in step 4.

6.3. 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 a 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 a reaction vial using a disposable 0.3 mL pipette.

WET SURFACES

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

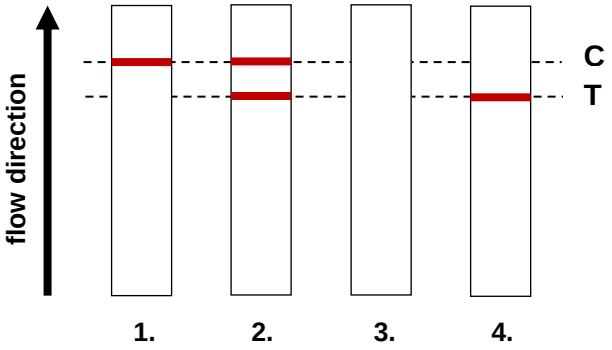
7. ASSAY PROCEDURE

1) Prepare samples as described above.
2) After transfer of the sample to the incubation vial add cap and shake for 15 seconds. Make sure that the biotinylated antibody is completely dissolved.
3) Incubate for 3 minutes.

4) Remove cap and place one strip into the vial. For proper strip orientation make sure that the arrows on the cover foil point downwards.
5) Incubate for 5 minutes.
6) Remove the strip from the vial and evaluate immediately.

8. EVALUATION

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



1. **Negative:** visible control (C) line, no test (T) line
2. **Positive:** visible control (C) and test (T) lines
3. **Invalid:** neither control (C) and test (T) lines visible
4. **Invalid:** no control (C) line and visible test (T) line

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 has to be compared with the different increments of the colour card. Results lower than increment 3 should be treated as negative. Results according increment 3 or higher should be treated as positive. 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 *RapidScan ST5* lateral flow reader is possible. For further information, please contact Gold Standard Diagnostics Budapest.

9. PERFORMANCE

All performance data was evaluated based on *Sigma-Aldrich Gliadin from wheat, Cat. no. G3375*.

9.1. 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 the 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 not cross-reactivity could be detected:

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

The following cross-reactions were determined:

Arrowroot	0.0008%

9.3. High-dose-hook Effect

Reduced or absent signals can occur in case of very high concentrations. The test gives valid results up to a concentration of 1000 ppm for food samples, according to 2.6 mg/cm^2 for swabs and 67 mg/L for rinse water samples.

9.4. Additional Performance Data

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

10. 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.