# SENSIS*trip* Gluten 20 Tests

## Lateral-flow Device for the Determination of Gluten in Food and as Cleaning Control Monitoring (Cat.nr. HU0030118)

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|---|---|
| Sensitivity for food matrix | 4 ppm |
| Sensitivity for swabbing | 1.6 ng/cm$^2$ |
| Sensitivity for rinse water | 0.04 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 **Eurofins Technologies Gluten 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. 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 **Eurofins Technologies Gluten** test is based on the principle of immunoassay. Gluten containing sample is given into a reactions 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. Extraction 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.

### 4. CONTENTS OF THE KIT

The kit contains components and reagents for 20 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.

1) Test strips, 20 pcs in tube with desiccant stopper.
2) Reaction vials, 20 pcs.
3) Extraction tubes with caps, 20 pcs.
4) Dilution tubes, 20 pcs
5) Dilution buffer, 60 mL, ready-to-use.
6) Disposable pipettes, 0.3 mL, 20 pcs
7) Disposable pipettes, 3 mL, 2 pcs
8) Disposable spatulas, 20 pcs
9) Swab sticks, 20 pcs
10) Evaluation card
11) Tubes and vials racks
12) Instruction manual

### 5. ADDITIONAL REAGENTS (NOT PROVIDED)

1. Ethanol (50%)


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## 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.

### 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 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 vial using a disposable 0.3 mL pipette.
7. Close dilution tube with cap and shake for 1 minute.
8. Add 3 mL of dilution buffer to the sample by using the second of the disposable 3 mL pipettes.
9. Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial using the same 0.3 mL pipette as in step 6.

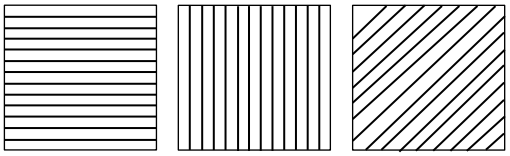
### RINSE WATER

1. Adjust the pH of the sample to 7 (+/- 0.5).
2. Transfer 0.3 mL of sample into a reaction vial using a disposable 0.3 mL pipette.

### 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 extraction solution into an extraction tube by using the disposable 3 mL pipette.
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 a reaction vial using a disposable 0.3 mL pipette.

*WET SURFACES*

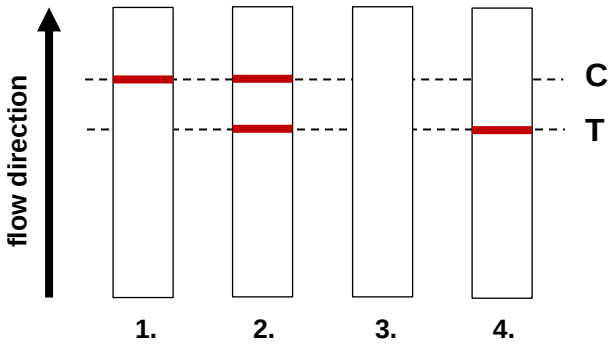
Apply same method as described for dry surfaces without prior need to moisten the swab.

## 7. 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 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.

## 9. PERFORMANCE

### *Sensitivity*

LOD (Gluten) of the SENSI*Strip* lateral-flow test is 4 ppm for food matrix, 0.04 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.

### *Cross-reactivity*

For the following foods not cross-reactivity could be detected:

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

### *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 2.6 mg/cm$^2$ for swabs and 67 mg/L for rinse water samples.

### *Additional Performance Data*

Additional data can be found in the corresponding validation report of the product, which can be inquired at Eurofins Technologies.