Disp&FLOW®–Gluten

Rapid test for the detection of gliadin and related prolamins

N° cat. : BIO.040.1

Number of test : 1

Rapid immuno-chromatographic test for the qualitative or semi-quantitative determination of the presence of gliadin and related prolamins (hordenin and secalin) in foods. The Disp&FLOW - Gluten test has been designed to detect target antigens in two main types of specimens:

1. Solid food samples / solid food products / kitchen utensils / technical surfaces dedicated to the cutting, processing and storage of food products.

2 . Samples more or less liquid such as: dessert creams; spreads; rinsing water from food preparation tools, rinsing water from kitchen utensils.

How it works

Gluten is a mixture of proteins combined with starch present in the endosperm of most cereals. Gluten is divided into two groups: prolamins and glutenins. Proteins in the prolamin family are the source of allergies, coeliac disease, skin diseases and central nervous system disorders. The prolamins found in wheat (alpha gliadin), rye (secalin) and barley (hordenin) are the most harmful.

Celiac disease affects between 0.2% and 1.2% of the world population, with a certain proportion of benign cases remaining undiagnosed. Gluten allergy is characterized by a variety of symptoms ranging from mild oral allergy such as urticaria to severe and potentially fatal systemic reactions such as bronchial asthma or anaphylactic shock. Gluten allergies are common among food allergies, affecting some 0.1% to 0.2% of the world population, and more specifically children. Strict elimination of gluten from the diet is the most effective prophylaxis.

In the United States, the Consumer Protection Act (FALCPA) requires special labelling when the gluten content exceeds 20 ppm.

In the European Union, cereals containing gluten are included in the list of food allergens drawn up by the European Food Safety Authority. Food products likely to contain gluten may only be labelled 'gluten-free' when the gluten content is less than 20 ppm, and 'low gluten' when the gluten content is less than 100 ppm.

In other countries, such as Australia, legislation requires much lower levels of gluten (2ppm) for foods labelled 'gluten-free'.

The Disp&FLOW - Gluten test is based on the principle of rapid immunochromatography on a strip (lateral flow migration). The target antigen found in the sample is absorbed by the strip and then recognized by specific antibodies that are free to move and conjugated to colored microparticles. Once formed, this complex migrates along the strip to a highly focused area where it meets another specific antibody attached to the support. The accumulation of microparticles forms a colored line that indicates a positive result. The presence of a second control line guarantees that the test is working correctly.

The Disp&FLOW- Gluten test detects gliadin and related prolamins (hordenin and secalin) accounting for around 50% of the total gluten content.

Test specificity and sensitivity

The Disp&FLOW - Gluten test uses a pair of monoclonal antibodies directed against various forms of gliadin and related prolamins (hordenin, secalin).
The Disp&FLOW - Gluten test does NOT detect the antigens related to the leguminous plants (including lupin), rice (orzenin), maize (zenin), millet (panicin), buckwheat, amaranth, quinoa and is weakly reactive to oats (avenin). The characteristics of the test, assessed by the limit of detection (LOD) and the dynamic reading range (ROD) for different prolamins, are shown in the table below.

Cereal	Prolamin	LOD (ppm)	ROD (ppm)
Wheat	Gliadin	**2**	**2 – 2 000**
Barley	Hordenin	**2**	**2 – 40 000**
Rye	Sécaline	**3**	**3 – 40 000**
Oat	Avenin	**200**	**200 – 400 000**

IMPORTANT NOTES: The Disp&FLOW - Gluten test is not suitable for beverages or prepared sauces due to the absence of high molecular weight components (either as a result of specific chemical treatment [acid hydrolysis] or as a result of enzymatic degradation during processing).

The sensitivity of the Disp&FLOW - Gluten test is calculated considering the target antigen contained in the solid material to be tested and after preparation, with a final solid/liquid ratio (weight/volume) of 1/10. The sensitivity of the test decreases when the target antigen is mixed with fat-rich ingredients (cream, butter, vegetable oils) or when food is heated (cooking).

Further data on the performance (sensitivity and specificity) and processing of the matrix can be requested from info@biotem.fr. If the test result is a weakly colored line or is difficult to interpret, BIOTEM recommends re-testing the sample with another kit or using a different method, such as quantitative ELISA or PCR.

Kit contents

The Disp&FLOW - Gluten test contains the following items:

- A test strip packaged in a hermetically sealed aluminium pouch containing a desiccant
- A tube containing 10mL of extraction buffer (white cap)
- A tube containing 4mL of dilution buffer (blue cap)
- A sample collection swab
- 250µL transfer pipette with long stem (for liquid samples only)
- 100µL transfer pipette with medium stem
- 20 µL transfer pipette with short stem
- Instructions for use
- Digital measuring spoon (optional)

Storage and stability

- The kit should be stored between +2 and +30°C in a dry atmosphere away from direct sunlight.
- **The strip should not be frozen and should be kept in its hermetically sealed pouch.**

The kit must be used before the expiry date indicated on the packaging.

Equipment required but not supplied

- Sampling spatula, preferably disposable
- Mortar or blender
- Micropipettes (optional)
- Centrifuge (optional)
- Precision scale

Précautions

- The kit components are for *in vitro* use only.
- The kit may be used up to its expiry date if stored under the conditions indicated.
- Do not use the test after its expiry date.
- The heat-sealed pouch containing the test strip should be stored at room temperature (+2 and +30°C).

- All handling associated with the use of this test must be carried out in strict compliance with the conditions for non-contamination of samples; in particular, gloves must be worn during handling.
- Do not use the test if the aluminium pouch has been found torn or cut open in the box.
- **The strip must be kept in its hermetically sealed pouch** (strips are very sensitive to high humidity) - do not use a strip more than 10 minutes after opening the pouch.
- Care should be taken when opening this pouch (see point 1 of the 'Test procedure' section) to avoid cutting or damaging the strip.
- Once the pouch has been opened, the strip should be handled by its upper colored part. Do not touch the central part of the nitrocellulose strip or its absorbent end directly.
- Only use the tube containing the extraction buffer supplied in the kit. Never use components from different kits.
- Take care not to immerse the strip deeper than the line under the arrows.
- The Disp&FLOW - Gluten test contains only single-use components; do not reuse.

Waste disposal

- Dispose of all used consumables in accordance with biomedical waste regulations.
- All users are responsible for managing the waste they produce and for ensuring that it is disposed of in accordance with applicable regulations.

Sample preparation procedure

Samples should be brought to a temperature between +18°C and +35°C; analysis of colder samples reduces the sensitivity of the test; analysis of warmer samples is not possible due to the risk of degradation of the antibodies present in the strip. Make sure that the material to be tested is a mixture of all the ingredients that make up the final solid food product.

For solid materials, utensils or other surface tests, we recommend the following procedure**:**

1. Using clean tools (preferably disposable), cut a small piece (5 to 10 g) of the material to be tested using the measuring spoon (optional) or a precision scale.
2. Crush the food sample using a blender or mortar until a homogeneous paste is formed. **Caution: Rinse the crusher thoroughly before each new sample to avoid cross-contamination.**
3. Using the measuring spoon (optional) or a precision scale, accurately insert 1 g into the white-capped tube containing the 10 mL extraction buffer.
4. For utensils or other surface tests, use the swab provided and pass it over the surface of the object to be analyzed in a criss-cross motion, first in one direction, then in the other, then diagonally. Then insert the swab into the test tube and shake it for 10 to 15 seconds in the extraction buffer.
5. Hermetically seal the tube with the white stopper.
6. Vigorously shake the tube manually or using a vortex at maximum speed for 30 seconds.
7. Incubate for 30min at 20-25°C, periodically shaking the tube manually or by vortexing.
8. If necessary, centrifuge the extract for 5 minutes at 200 x g or allow the sample to decant to remove as many suspended particles as possible.
9. NEVER use the extract undiluted in dilution buffer: Dilute the extract as follows with the dilution buffer contained in the white cap test tube:

For a sensitive determination (LODgliadin=**2 ppm**), remove precisely **200 µL** (using a laboratory pipette or by **aspirating 2 times the total volume from the medium stem transfer pipette supplied)** of the extract contained in the 10mL tube (white cap).

For routine screening (LODgliadin=**20 ppm**), accurately remove 20 µL (using a laboratory pipette or by aspirating 1x the total volume from the **short stem** transfer pipette supplied) of the extract contained in the 10mL tube (white cap).

Then transfer the chosen volume to the **4mL** dilution buffer supplied (tube with blue cap). Vortex or shake manually.

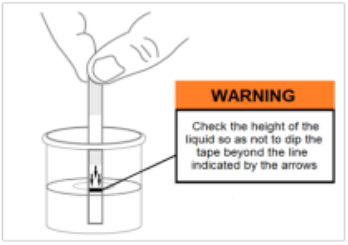
For liquid samples :

The test detection limit for liquid samples depends on their viscosity and turbidity.
If the sample is viscous and cannot reach the test zone by migration on the strip, it must be diluted in the dilution buffer. In this case, the sensitivity of the test must be adjusted by the dilution factor.
Cloudy specimens should be filtered through a textile or paper filter.

1. Shake the liquid test material vigorously, either manually or using a vortex at maximum speed, for 20 - 30 seconds.
2. Accurately insert 1mL of the liquid sample to be tested into the white capped tube containing 10mL of Extraction Buffer. If using the provided transfer pipette with long stem, transfer 4 times the total pipette volume of liquid sample into the tube containing 10mL of Extraction Buffer.
3. Hermetically seal the tube with the white stopper.
4. Vigorously shake the tube manually or using a vortex at maximum speed for 20-30 seconds.
5. Continue as in point 7 of the 'Sample preparation procedure / For solid materials'.

Test procedure

1. Bring the samples to a temperature between +18 and +35°C.
2. Remove the aluminium pouch (without opening it) and leave it at room temperature for 5 to 10 minutes.
3. Open the pouch containing the test strip, taking care not to cut it.
4. Grasp the strip by the top colored part and dip the other end vertically into the supernatant in the blue-capped test tube.
   Caution: make sure that the strip is not immersed too deeply - see diagram opposite. ➔

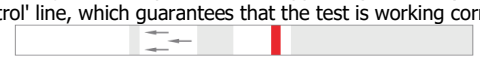


5. Soak the strip for 20 - 30 seconds and then place it on a CLEAN, horizontal surface; do not touch or move the strip for 10 minutes while the sample migrates.
6. Read the result and interpret it in accordance with the diagrams and instructions below.

Interpretation of results

The test is positive if 2 red lines appear clearly in the central area of the strip (the 'Test' line and the 'Control' line, see below). Do not take into account the order of appearance of the 2 lines or any nuances in color intensity.

The test is negative if only one red line appears (see below): this is the 'Control' line, which guarantees that the test is working correctly.



If the line 'Test'- and only it - appears (see below), the test cannot be interpreted, and no result is validated.

If no line appears (see below), the test cannot be interpreted, and no result is validated.

In the latter two cases, before starting again with another Disp&FLOW - Gluten test, it is important to ensure that all the instructions for preparation, storage and use of the test have been followed, as well as the expiry date.

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