

COD 14206	10 tests
Reagents for the determination of gluten in food samples	
Only for <i>in vitro</i> use in the laboratory	

GLUTEN R5 (food) flow through IMMUNOASSAY ON NITROCELLULOSE

GENERAL INFORMATION

Gluten is the main part of the protein fraction of cereals. 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). The disease is caused when toxic proteins in cereal gluten damage the absorptive areas of the small intestine and can only be reversed by strict adherence to a diet low in gluten. 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.

INTENDED USE

The Gluten R5 (food) Flow Through Test is an immunoassay on nitrocellulose for the screening of gluten in food samples. These qualitative tests utilise highly specific monoclonal Mendez R5 antibodies that allows small laboratory and non-technical/home users to detect the presence of low (mg/kg = ppm) levels of cereal gluten in food raw materials, part-processed foods and many finished food products quickly and with the minimum of equipment.

The Flow Through™ test range has been developed and manufactured by Bio-Check (UK).

CONTENTS AND COMPOSITION

The kit contains 10 bags each comprising:

Extraction Solution. 1 tube containing a yellow solution.

Diluent. 1 tube containing colorless solution.

Visualisation Solution. 1 tube containing a pink colored solution.

Scoop.

Spin tube.

Pipette. For 150 µL.

Cotton bud.

Test Unit. In a protective foil pouch.

STORAGE AND STABILITY

Store at 2-8 °C. Each component is stable until the expiry date marked in the label.

Indications of deterioration:

- Reagents: Presence of particulate material or turbidity that can not be redissolved by gentle heating.
- Test Unit: rips on the sealing bag, presence of spots in the membrane before performing the assay. Use only if the desiccant in the foil pouch is colored yellow/pale green.

REAGENT PREPARATION

All the components are provided ready to use.

PRECAUTIONS

- The components contain no toxic or harmful additives/preservatives and are completely safe if used as described in these instructions.
- Do not use components past expiration date and do not intermix components from different serial lots.
- Once the foil pouch has been opened, use the Test Unit within the working day/shift.

SAMPLE TREATMENT

A homogeneous sample has to be obtained from a representative part of the compound.

1. **Fine powders, flours, smooth pastes and liquids** need no preparation.
2. **Unprepared food samples:** finely chop, grind or mill the food sample to obtain a homogeneous consistency. The smaller the particle size, the more efficient the extraction will be and the more accurate and consistent the test result.

PROCEDURE

1. Remove parts from bag. Invert (separately) the three tubes containing liquids several times to ensure they are well mixed (Notes 1, 2).
2. Weigh 0.50 g (\pm 0.02 g) of sample into an Extraction Solution tube using the handle end of the scoop provided. Alternatively, fill the 0.5 mL scoop (approx. 0.25-0.50g depending on sample density) with sample. If testing fine powders, ensure that they are firmly compressed into the scoop. Carefully add sample to the Extraction Solution by inverting the scoop in the mouth of the tube and tapping to empty the contents into the liquid (Note 3). Replace cap.
3. Mix extract by shaking tube vigorously for at least 2 minutes to maximise recovery of gluten from the sample (Note 4). Allow tube to stand for at least 10 minutes to help extract any gluten. Shake tube vigorously again for at least 2 minutes.
4. Let stand for 20 minutes to allow the extract to separate until a clear layer is seen above the solid food sample. Alternatively, centrifuge the tube directly. You can also fill the spin tube about two thirds full with extract, click down the cap and spin the tube(s) at 2,000 x g or above for about 5 minutes.
5. Fill the self-measuring pipette with some of the clear, yellow food extract by squeezing the bulb tightly, placing the end of the pipette into the solution, below any fat layer, and releasing the bulb until solution over-fills the measuring tube and flows into the lower bulb.
6. Remove cap from the Diluent tube. With the self-measuring pipette in the Diluent liquid tube, squeeze the bulb to add sample extract to the Diluent liquid. Recap Diluent tube and invert several times to mix gently.
7. Tear open foil pouch and remove the Test Unit. If necessary, label with sample information/number.
8. Remove cap from Diluent tube containing food extract. Slowly and carefully pour the diluted extract directly into the well of the Test Unit.
9. Allow diluted extract/swabbing solution to absorb into the unit. This should take 3-5 minutes (Note 5).
10. Using the round ends of the cotton bud, gently clean the surface of the round test area at the base of the well and then use the sides of the cotton bud to clean and dry the rim and top of the well.
11. Remove the cap from the Visualisation Solution tube, invert and slowly and carefully add the contents directly into the well of the Test Unit. Allow solution to absorb into the unit. This takes 2-3 minutes.

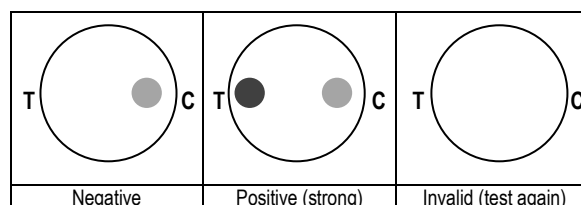
READING

Read the test result immediately in good light and at your normal reading distance.

Negative result. A color spot appears only in the side "C" of the well.

Positive result. Two color spots appear: one in the side "T" and another in the side "C" of the well. A sample containing 20-100 mg/kg gluten should give a "T" spot that is weaker than or similar in intensity to that of the "C" spot. The darker the test (T) spot the more gluten is present in the sample being tested (Note 6)

Invalid result. Absence of color spot in the side "C". Retest the sample using a new bag.



It is recommended that some additional laboratory testing of samples is performed using tests with higher sensitivity (e.g. Biosystems Gluten ELISA kits, cod 14119) to confirm results and help ensure that your gluten free processes are effective.

ASSAY CHARACTERISTICS

- Cut-off: 4 mg/kg (ppm) (Note 7)
- Specificity: The tests use well characterised Mendez R5 monoclonal antibody that predominantly detects alpha-, gamma- and omega-gliadins, secalins and hordeins as a marker of total gluten from e.g. wheat, rye, Durum, kamut, spelt and (with lower activity) barley. They have been tested in this format against a panel of FAPAS proficiency testing samples, gluten free raw materials, baking mixes and finished products (both wheat-derived and wheat-free).

VALIDATION

A Customer Validation Report is available on request. The performance of the tests has been assessed compared to confirmatory laboratory ELISA methods.

NOTES

1. If Extraction Solution is not completely clear, warm in the hand or in "hand hot" water to re-dissolve.
2. If running more than one test, it is recommended to write sample information/reference number(s) on Extraction and Diluent Liquid tube labels.
3. For sauces and other viscous liquids the bowl of the scoop can be broken off by trapping it in the tube with the cap and bending the handle sharply downwards. The bowl will drop into the solution. Mix thoroughly to disperse the sample adhered to the bowl.
4. Gluten is harder to extract from processed foods than from simple flours/pastes. To improve the extraction process heat the tube by placing it into a heater block at 55-60°C or into hot (55-60°C) tap water. Leave for 15 minutes, mixing the tube occasionally.
5. If the extract is particularly viscous or contains particulates it may absorb more slowly; if so proceed to the next step after 8-10 minutes i.e. pour off excess liquid or absorb it with a tissue before all of it has been absorbed.
6. Samples containing very high levels of gluten will stain the background of the test area pink, often unevenly. In such cases a strongly colored Test spot and a Control spot should also be observed. If background/patchy red coloring is observed and no clear Control spot is seen, repeat the test adding only two drops of extract from the pipette to check whether the sample has a very high gluten content.
7. Limit of detection (LOD) determined by using Prolamin Working Group (PWG) gliadin reference material. The LOD level of GFT-R5 is low micrograms per gram of food but varies significantly depending on the composition and (particularly thermal) processing history of the material being tested. LOD also depends upon, amongst other factors: sampling procedure; sample preparation/homogeneity; structural integrity of cereal gluten; its solubility during extraction and reactivity in the test. It is strongly recommended that GFT-R5 performance is assessed by validation in comparison with quantitative laboratory methods.