

Gluten R5 FlowThrough™ test



**Qualitative test for on-site detection of gluten
in raw materials, part-processed and finished foods**

PLEASE READ INSTRUCTIONS BEFORE USE

Document CP044 – REV01

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GLUTEN R5

Qualitative Onsite Tests

Extraction: 40 min.
or less

Test: 6 min.

Total Test time: 45 min.
or less

Bio-Check's Quality Management System is ISO 9001:2008 approved for development and manufacture of test kits for the detection of contaminants and adulterants in food products.

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Gluten R5 FlowThrough™ test

For on-site detection of cereal gluten in food



These instructions (IFU) are applicable to 1 (R6095), 5 (R6096) and 100 (R6097) test pack(s).



- Please inspect all components and read IFU carefully before proceeding.
- History of IFU amendments can be found on page 7.
- For single use within their expiry date; open only when ready to start.
- All components are non-toxic and safe to use as directed.
- Store kits between 2°C/36°F to 8°C/46°F. Use between 15°C/59°F to 25°C/77°F.

Intended Use of GFT

Gluten R5 FlowThrough™ (GFT-R5) tests can help verify low gluten levels in raw materials prior to use in manufacturing and the 'gluten-free' status of part-processed or finished products. GFT tests have been designed to be simple enough for testing to be performed outside the laboratory with minimal equipment, yet sensitive enough to confirm that gluten levels in a wide range of food commodities and products are likely to comply with international Codex Standard 118:1979 (2015) and local regulations e.g. less than 20mg/kg gluten content under Regulation (EU) 1169/2011 and 609/2013 for "gluten free" foods. Periodic QC checks, as well as additional, confirmatory laboratory testing (e.g. using Bio-Check's Gluten-Check™ ELISA kits) must be performed to help confirm GFT R5 testing results and that overall gluten-free control processes are effective.

GFT-R5 Detection Limit

These qualitative tests utilise highly specific monoclonal Mendez R5 antibodies to detect gluten in food (see page 7). The Limit of Detection (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 (see P.7. and Validation report). 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. For further information regarding fitness for purpose and validation of GFT-R5 tests, contact Bio-Check.

Components for a single GFT-R5 test

	Quantity
0.5cc plastic sample scoop	1
Push-cap tube containing YELLOW Extraction solution	1
Plastic spin tube with lid	1
Self-measuring pipette (100µL/0.100mL)	1
Screw-cap tube containing COLOURLESS Diluent liquid	1
Test Unit (TU) in foil pouch with desiccant	1
PINK Colour Reagent in BLUE capped tube	1
Cotton bud	1



Overview of the GFT-R5 test

GFT-R5 tests follow a five-part procedure. It typically takes about 10 minutes to perform a GFT-R5 test (plus up to 40 minutes for preparation/extraction). After the sample is taken and prepared, it is extracted in yellow Extraction solution (optional heating step and/or centrifugation). A portion of the sample extract is then diluted and added to the Test Unit; any gluten present binds to a Test spot (T) on the left hand side of the test area. The presence of gluten is indicated - after the addition of a pink Colour Reagent – by formation of a pink spot at 'T'. A pink Control (C) spot also appears on the right hand side of the test area to indicate that the GFT-R5 test has worked properly.



Please see page 4 for details

Validation

A Validation Report is available upon request; it includes test results for FAPAS Proficiency samples, providing an assessment of performance compared to confirmatory laboratory ELISA.

Precautions

- Store the kit between: $2^{\circ}\text{C}/36^{\circ}\text{F}$ $8^{\circ}\text{C}/46^{\circ}\text{F}$ Use between: $15^{\circ}\text{C}/59^{\circ}\text{F}$ $25^{\circ}\text{C}/77^{\circ}\text{F}$ Single use:
- Test components should only be used within their specified expiry date.
- Test components should only be used as provided i.e. as a complete set.
- Once the foil pouch has been opened, use the Test unit within the working day/shift.
- Only use the Test unit if undamaged & the desiccant is coloured yellow/pale green.
- Dispose of used test components in normal waste.

Warranty

Bio-Check (UK) Ltd ('Bio-Check') supplies products under its Terms and Conditions, as published on its website, which will have been brought to the attention of all customers prior to ordering and further notified in writing with the invoice for the goods. Bio-Check warrants products supplied ('Product' or 'Products') against defects in materials, workmanship or performance, when stored and used exactly in accordance with the applicable Instructions For Use, up to a Product's expiration date. If a customer establishes that Product does not conform to this limited warranty, Bio-Check shall, at its option, replace such of the Products with similar Products or allow the customer credit for the Product's invoice value but Bio-Check will have no further liability to the Customer. Bio-Check makes no other warranties expressed or implied including, but not limited to, any implied warranties or merchantability or fitness for any particular purpose. Bio-Check does not warrant against damages or defects arising in shipping or handling, or against accident in use of, or improper or abnormal use of, Product. Bio-Check shall not be liable for damages of any kind, including special or consequential damages, or expenses arising directly or indirectly from the use of Products or the interpretation by users of results obtained when using Products.

Gluten R5 FlowThrough™ Procedure

For on-site detection of cereal gluten in food



A robust, well designed sampling plan will increase the chance of detection. Ensure that sample inspection points chosen have been risk assessed. **IT IS IMPORTANT THAT GFT HAS BEEN VALIDATED WITH EACH SAMPLE TYPE TO BE ANALYSED TO ENSURE THAT GFT-R5 IS FIT FOR PURPOSE.** For ease of handling, some test components are coloured. The Extraction solution is yellow in a non-coloured cap tube; Colour Reagent is pink with a blue coloured cap. Label all tubes and Test Units with a permanent marker to aid identification when performing the test.

Sampling & sample preparation



<5 minutes



Sampling helps obtain a **representative** amount of material from an original 'lot'. Sample preparation helps ensure that the material is **homogeneous**. It is highly likely that gluten contamination will **not** be spread evenly throughout the lot; therefore, both steps must be optimised to help ensure that gluten is more likely to be detected. Optimised combinations of sampling, sample preparation and testing frequency help ensure that testing variations are minimised. Consideration of sampling sites, sample size, sample numbers and sample particle size are all key aspects of a risk-based sampling programme.



1. IMPORTANT Sampling is a critical step

Ensure that samples tested are representative of the original lot, taking care to avoid cross-contamination.

2. Sample prep

- Fine powders, flours, smooth pastes & liquids require no preparation.
- For all other foods: finely chop, grind or mill the food sample to obtain a homogenous consistency and small particle size.

3. Prepare test

- Remove sufficient test components and label for testing. Ensure all tubes are well mixed.
- **If Extraction solution is refrigerated see below ***

* **IMPORTANT: Refrigerated Extraction solution (single/5-tests) forms a precipitate.** To re-dissolve before use, warm tube in the hand/hot water and mix thoroughly until solution is a clear yellow colour.

For best results some optional equipment may be useful when performing this test:

- A food processor / mortar & pestle may be required to make the food sample homogenous
- A two place balance is preferred for accurate weighing out of food samples (0.5g±0.02g)
- A vortex mixer or shaker is best for mixing the food extracts and reagents
- A heater block is useful for heating processed food samples during extraction
- A mini-centrifuge to spin down un-dissolved food greatly shortens test times

Extract sample

<15 minutes

- i** If performing more than one test, label all tubes with your sample reference. If the sample is not weighed, ensure the scoop is filled level with the rim of the bowl.



4. Test portion*

- EITHER: place Extraction tube on balance; tare to zero. Weigh 0.5g(±0.02g) into the tube using scoop.
- OR: fill the scoop with sample, & carefully add to Extraction tube.
- **Recap tube.**



5. Shake tube

- Mix extract by shaking the tube vigorously for at least two (2) minutes.
- This helps dissolve gluten from the sample ready for testing.



6. Extract

- EITHER: allow to stand for 10-15 minutes.
- OR:
- For processed foods heat the tube at 55-60°C for 15 minutes.
- **Shake again for 2 minutes.**

* For sauces, viscous liquids etc. the bowl of the scoop can be broken off by pushing against the inside wall of the tube and bending the handle sharply downwards. The bowl will drop into the solution and will aid the mixing in the next step.

Dilute Sample Extract

<25 minutes



7. Allow extract to separate

- EITHER: allow to stand for 20 minutes until a clear, upper layer is seen.
- OR: fill the spin tube 3/4 full, cap and spin for 5 minutes.



8. Pipette extract

- Using the pipette provided carefully remove some of the clear yellow extract below any surface fat layer, by tightly squeezing the bulb & releasing until the solution overfills the measuring tube and flows into the lower bulb.



9. Dilute extract

- Remove cap from Diluent tube & insert pipette.
- Squeeze pipette bulb to add extract to tube.
- **Recap tube**
- **Mix gently.**

Add diluted sample extract

3 minutes



Desiccant sachet in foil pouch should be yellow/pale green in colour.

Read GFT-R5 test result after Colour Reagent has been absorbed into the Test Unit (TU).

Record any **clearly visible** pink spot on left of the TU as a DETECTED response.



10. Open foil pouch

- Remove Test Unit (TU) from pouch and inspect before use.
- Label TU if more than one test is to be performed.



11. Add extract to Test unit

- Carefully pour the diluted Extract into the well of the TU.



12. Allow to absorb

- Wait until the diluted Extract is **completely absorbed**.
- If it takes longer than 10 minutes pour/blot off excess liquid.
- **Clean the test area ***

* **IMPORTANT:** When diluted extract has absorbed into the test unit, clean surface of test area **CAREFULLY** with a cotton bud.

Add pink Colour Reagent

3 minutes



Invert pink Colour Reagent tube(s) several times to mix before use.



13. Add Colour Reagent

- Remove cap from pink Colour Reagent tube.
- Carefully pour contents into the well of the TU.



14. Allow to absorb

- Wait about 3-5 minutes until the pink Colour Reagent is completely absorbed into the well of the TU.



15. Read

- Read the test response within one hour using the guidance notes below.

Read

Total GFT-R5 test time: 45 minutes or



Read the GFT-R5 test result **in good light** and at your normal reading distance within an hour of finishing the process. The appearance of a **CLEARLY VISIBLE**, pink Test spot on the left of the test area (T) **regardless of intensity**, indicates that gluten is detected.

The darker the Test spot the more gluten is detected in the sample extract being tested:



NOT DETECTED



DETECTED (WEAK)

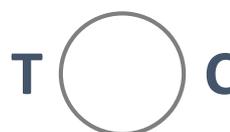


DETECTED (STRONG)

A pink Control spot of medium intensity should ALWAYS appear on the right hand (C) side of the test area; this indicates that the GFT-R5 test result is VALID, the test has been performed correctly and all reagents are functional. When gluten levels are very high in the sample being tested, the whole of the test area may be stained pink but a T and C spot should always be seen.



INVALID RESULT



INVALID RESULT

If a Control spot does not appear, the result is INVALID and must be repeated. The spots are stable for up to 1 hour after completing the test but change in appearance as the unit dries out, which may lead to incorrect result interpretation.

GFT-R5 Test reagents & Validation

GFT-R5 tests use well characterised antibodies as described by Mendez (Eur J Gastroenterol Hepatol. 2003 May;15(5):465-74) that recognise the potentially coeliac-toxic epitope QQPFP most strongly. R5-based laboratory ELISAs have been collaboratively tested and widely used for gluten determination for many years. Mendez R5 monoclonal antibody was raised against a secalin extract; it predominantly detects alpha-, gamma- and omega-gliadins, secalins and hordeins. These protein families serve as a marker for total gluten from wheat (including Durum & Khorasan), rye, spelt, kamut and (with slightly higher reactivity) barley. The GFT-R5 test does not react with non-gluten containing ingredients: maize, oats, rice and soya. More highly processed foods (e.g. pasta, biscuits) may be difficult to extract such that GFT-R5 Limit of Detection may be somewhat raised. In such samples, heating during extraction is strongly recommended to improve the solubility of gluten. Otherwise, Bio-Check's Gluten-Check™ ELISA may be more appropriate. For advice on analysing beer or difficult samples please contact Bio-Check (UK).

GFT-R5 Extract storage & ELISA testing

It is preferable to test the separated extract within the working day/shift, however, it can be stored at **room temperature** (around 20°C/68°F) for up to two days prior to testing if necessary. If required, the sample extract can also be tested using Bio-Check's Gluten-Check™ ELISA (after at least a 1/20 dilution).

Document changes

The most recent changes to these instructions for use (IFU) are recorded below. Please check this section to ensure you are familiar with the latest recommendations. Bio-Check welcomes suggestions to improve the IFU content and the products it describes.

Record of changes: Document No. CP044

REV01 (September 2016): New product

Contact us

Please contact us for further assistance:

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