

GLUTEN R5 FlowThrough™ for Food Samples

VALIDATION REPORT



REF No: **R6095** (1 Test)
 R6096 (5 Tests)
 R6097 (100 Tests)

Intended use

Gluten FlowThrough™ R5 (GFT-R5) for Food Samples can help validate and verify that allergen control procedures designed to ensure low gluten levels are effective. They can be used in the food manufacturing environment to help ensure the 'gluten-free' status of raw materials, part-processed and finished foods.

A robust, well designed and appropriately validated sampling & testing regime, which encompasses the use of confirmatory laboratory techniques helps to increase the level of protection.



It is important that the use of GFT-R5 for Food Samples has been validated by the user to ensure that it is 'fit for purpose'.

Performance characteristics

Parameter	Description	Section
Sensitivity	The lowest concentration that can be detected.	1
Selectivity	The extent to which a method can determine particular analytes.	2
Specificity	The effect of any interfering substances.	3
Repeatability	The agreement between results under repeatability conditions.	4
Robustness	The effect of changes to operational parameters on the results.	5
Stability	Recommended storage conditions and duration.	6

1. Sensitivity

Method 1: PWG* gluten solution was spiked into the Diluent/Extraction solution mixture and tested using GFT-R5 Food:

Tested	Gluten Level in Diluent	Equivalent in Product tested	Test Response
PWG*	30.0 µg	2,400 ppm	Positive
PWG	0.50 µg	40 ppm	Positive
PWG	0.10 µg	8 ppm	Positive
PWG	0.05 µg	4 ppm	Positive
No addition	None	None	Negative

* Prolamin Working Group (PWG) gliadin reference material produced under PWG guidance and handled as per the accompanying Instructions For Use.

Method 2: PWG gliadin solution, equivalent to a level of ~40ppm gluten in the tested product, was spiked into extracts of twenty-two food ingredients and tested using GFT-R5 Food.

Almond	Cornflour	Oats	Quinoa
Amaranth	Egg White	Paprika	Rice
Beef Protein	Egg Yolk	Peanut	Sesame
Brazil nuts	Haricot beans	Pine nut	Tapioca
Buckwheat	Lupin	Potato	Walnut
Coconut		Milk Powder	

Results: All spiked extracts produced clear positive results with similar response levels (grades 3-4 for “T” spot intensity):

Method 3: Two FAPAS Proficiency Scheme Positive and two Negative samples were mixed in different proportions to simulate a true gluten positive food matrix at different levels. The mixtures were extracted and tested using GFT-R5 Food.

Results: Positive results were seen at or above ~5ppm:

FAPAS 15ppm (1:1)	FAPAS 15ppm (1:4)	FAPAS 20ppm (1:1)	FAPAS 20ppm (1:4)
(~7.5 ppm)	(~3 ppm)	(~10 ppm)	(~4 ppm)
POSITIVE	POSITIVE	POSITIVE	NEGATIVE

Method 4: A total of twenty gluten free finished products (ten pizzas; five ready meals; five different cakes including iced and chocolate) were mixed with small amounts of an “incurred” (processed) food sample containing gluten to simulate a true gluten positive matrices at levels of ~20-25 ppm and ~8-10ppm. The food mixtures were then extracted and tested using GFT-R5 Food.

Results: Positive results were seen in all spiked samples.

Method 5: A total of eighteen Positive and Negative Q.C. samples (fifteen FAPAS Proficiency Scheme samples with consensus values between 15 ppm and 136 ppm and three in-house Q.C. materials including one with a high gluten level) were extracted and tested using GFT-R5 Food.

Results: Compliant results were seen for all samples except one FAPAS chocolate cake mix with a “Negative” designation, which gave a positive GFT-R5 result; this sample returned values between 2 ppm & 5 ppm by ELISA.

Conclusions:

The GFT-R5 for Food Samples is capable of detecting below 5-10 ppm gluten in a wide variety of food products and test response is not affected by a variety of common food ingredients.

2. Selectivity

The **Mendez R5** monoclonal anti-gliadin antibody used in this kit reacts to gluten-equivalent cereal proteins from a number of cereal species. Several cereals were tested in the GFT-R5 Food test by extracting flours as described in the Instructions For Use and further diluting the extracts to levels equivalent to ~20ppm of gluten-like protein. Their relative reactivities at this level were as follows:

Cereal	Reactivity
Wheat	***
Rye	***
Spelt	***
Barley	***
Kamut	**
Durum Wheat	*

Antibody reactivity: R5 antibody is directed towards a particular amino acid sequence in cereal storage proteins (QQFPF) but recognises structurally similar sequences. Such sequences occur in multiple positions within α , β and γ wheat (gliadin) proteins, as well in barley (hordein), rye (secalin) and related cereal proteins. R5 shows a high degree of reactivity to γ gliadins, which appear to be especially resistant to heat. The QQFPF sequence is also found in α -gliadin 33mer, which is reported to be a significant immuno-toxic and protease-resistant peptide in Coeliac Disease pathogenesis.

3. Specificity

The following commodities, ingredients and retail samples DID NOT react in the GFT-R5 Food test when extracted, diluted and tested as per the Instructions For Use:

Commodities, Ingredients & Retail samples			
Aduki beans	Cornflour	Macadamia	Pumpkin seeds
Alfalfa seeds	Cumin	Maize	Quinoa
Almond	Currants	Malt vinegar	Raisins
Amaranth	Drinking chocolate	Millet	Red kidney beans
Amica lentils	Egg White	Mung beans	Rice flour
Apricot kernels	Egg Yolk	Mustard	Rice, Basmati
BBQ Beef Seasoning	Fennel	Nutmeg	Rice, brown
Beef	Fish protein	Oats	Sesame
Beef Protein	Fish sauce	Paprika	Sheep, meat
Blackeye beans	GF Flour	Pea protein	Skim milk (cow's) pwdr
Brazil nuts	GF vegetable gravy mix	Peanut	Skim milk (sheep) pwdr
Buckwheat	Ginger	Peas, green split	Soya flour
Butter beans	Haricot beans	Pecan	Soya sauce
Cannellini Beans	Hazelnut	Pepper, black	Sugar
Casein	Horse	Pepper, white	Sultanas
Cashew	Instant coffee	Pork	Sunflower oil
Celery salt	Lactose	Pine nut	Sunflower seeds
Chicken	Lentils, green split	Pinto beans	Tapioca
Chicken Gravy	Lentils, red	Pistachio	Tea leaves
Chickpeas	Linseed	Poppy seeds	Teff
Chillies	Lupin	Potato	Walnut
Coconut	Lupin oil	Potato starch	Worcester sauce

Beers: Five beer samples, one labelled gluten free, two bitters and two lagers – added directly to Diluent tubes – returned expected results in the GFT-R5 Food test.

4. Repeatability

Conditions	Number of Tests	Sample	Results Compliant
Same operator	Five at each level	Zero	100%
Different days	On each day	Limit of Detection	100%

Positive “T” spots and “C” spots showed very similar responses during the testing (Internal QC grades 3-4 for the “T” and 8 for the “C” spots).

5. Robustness

The following changes to GFT testing conditions were made and the effects on Negative, LOD and HIGH gluten levels were observed:

Procedural change	Results
Test units kept at 2-8°C for 4 hours	All compliant
Test units kept at room temperature for 4 hours	All compliant
Test units kept at room temperature for 25 hours	All compliant
Test units kept at 30°C for four hours	All compliant
Tests carried out at 2-8°C	All compliant
Tests carried out at 30°C	All compliant
GFT-R5 result read up to one hour after testing	All compliant

6. Stability

Storage conditions	Assessment	GFT-R5 Results
Refrigerated at 2-8°C (12 months)	Based on historical studies and ongoing stability trial data	Comply

Warranty

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Document changes

LA055 REV01 (Feb-2017): New product.

Contact us

Please contact us for further assistance.

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