GLUTEN-CHECK™ ELISA KIT

For the quantitative determination of low levels of cereal **GLUTEN**

in food ingredients, uncooked/cooked foodstuffs etc.

INSTRUCTIONS FOR USE

READ CAREFULLY BEFORE PROCEEDING!

Cat. No. R6005/ 6/ 7

48- / 96-/ 192-WELL KITS

STORE REFRIGERATED (2-8°C – see Section 5.2.1)

QIS101 GLUTEN ELISA R6005_6_7 V13 A2009 August 2012

Recent Amendments:

*****IMPORTANT CHANGES – PLEASE READ CAREFULLY*****

1. **Revised Standards range (5-110PPM) and now supplied as 20X CONCENTRATES.**
2. **Gluten values more closely aligned to FAPAS Consensus.**
3. **Incubation times shortened to 20 + 20 + 20 minutes.**
4. **Change to 1/20 dilution ratio of extracted samples/Controls.**

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INSTRUCTIONS FOR USE: CONTENTS

1. **INTRODUCTION TO THIS TEST KIT**

1.1. True gluten intolerance or Coeliac Disease (CD) affects 1% or more of the EC, US & Australian populations; CD is caused when toxic proteins in cereal gluten damage the absorptive areas of the small intestine and manifests itself as e.g. intestinal pain, bloating, cramps, and in children as a failure to thrive. CD can only be reversed by strict adherence to a diet low in gluten.

1.2. Increasingly many people also perceive themselves, rightly or wrongly, to be food intolerant and may avoid wheat/gluten as a result.

1.3. These phenomena have led to a rise in the availability of "gluten-free" food products and the consequent requirement to monitor gluten levels in such products more closely.

1.4. Codex Standard 118 and recent EC Regulations define "**Gluten Free**" (GF) foods for PARticular NUTritional use (PARNUTS) as containing less than 20 Parts Per Million (PPM; mg/kg). PARNUTS foods above 20 PPM & below 100 PPM must be labelled "**Very Low Gluten**".

2. **INTENDED USES OF THE KIT**

2.1. The assay utilises mouse monoclonal antibodies against gluten proteins in an ELISA technique designed for the measurement of cereal gluten at low (mg/kg; PPM) levels.

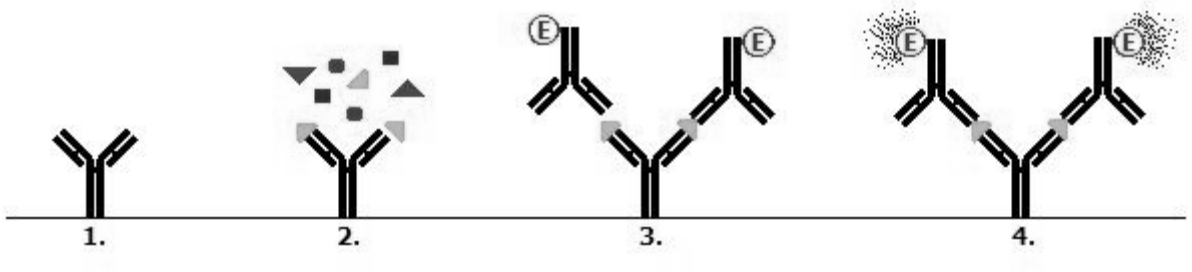
2.2. The range of the assay is nominally between **5** and **110 mg/kg** (PPM Gluten) if the stated extraction and dilution ratios are used but can be extended by altering the dilution ratio.

2.3. The specified Limit Of Detection (LOD) of the assay is well below **0.5PPM** gluten.

2.4. The assay can be used to test e.g. food raw materials/ingredients, environmental swab samples and part-processed/finished food products.

2.5. The assay, originally developed by Skerritt & Hill for high level gluten detection (Ref: JAOAC vol. 74, no. 2, 1991 pp. 257-264) detects the omega gliadin fraction of wheat as a marker of total gluten. Manufactured by in the U.K. under license from Vital Diagnostics, NSW, Australia.

3. **DIAGRAM OF THE ELISA METHOD:**



1. Antibody coated plastic micro-well.
2. Gliadin (▲) in ethanolic food extract binds to antibody on well.
3. HRP enzyme-labelled 2nd antibody binds in turn to gliadin bound to antibody on well.
4. Addition of TMB substrate, which develops blue colour in presence of HRP enzyme.

4. SAMPLE PREPARATION & ELISA OVERVIEW

4.1. Laboratory Sample/Kit Controls Preparation

- ⇩ **Prepare** Sample by grinding/chopping/blending until homogeneous.
- ⇩ **Add** 1 part Test Portion to 9 parts Extraction Solution (**1/10**).
- ⇩ **Extract** Shake 2 mins; **45 minutes @55°C** and mix during extraction.
- ⇩ **Mix. Separate** Shake 2 mins; 10 mins centrifuge OR 30 mins settle.
- ⇩ **Remove** a portion of the extract above the food pellet.
- ⇩ **Dilute 1/20** Standards & Sample/Control extracts in Diluent Solution 1 (1x).

4.2. ELISA Protocol

- ⇩ **Pipette** 100µL diluted Sample/Control Extracts & Standards into wells.
- ⇩ **Mix. Incubate** at room temperature for **20 minutes**.
- ⇩ **Wash wells** three times with wash solution 1X.
- ⇩ **Pipette** 100µL Anti-Gliadin HRP into wells.
- ⇩ **Mix. Incubate** at room temperature for **20 minutes**.
- ⇩ **Wash wells** four times with wash solution 1X.
- ⇩ **Pipette** 100µL TMB Substrate reagent into wells.
- ⇩ **Mix. Incubate** at room temperature for **20 minutes**.
- ⇩ **Pipette** 100µL Acid Stop Solution into wells.
- ⇩ **Mix. Read** wells at **450nm** wavelength.
- ⇩ **Calculate** mg/kg (PPM) Gluten results for all Controls/Samples.

5. **SAFETY/PROCEDURAL NOTES**

5.1. SAFETY

5.1.1. There are NO toxic ingredients/preservatives in any of the kit contents, therefore the kit is safe if used according to these instructions.

5.1.2. Acid Stop Solution 1 contains a relatively weak concentration of sulphuric acid (0.5M/1N): wear safety glasses; use with care; avoid splashing.

5.2. PROCEDURAL

5.2.1. **Store kit at 2-8°C.** To reduce the amount of refrigerator space required, **only the Gluten Standards, HRP & TMB reagents need to be stored cold.** Storage of Diluent/Wash Solution Concentrates at room temperature reduces the formation of crystals (which must be re-dissolved prior to use).

5.2.2. Users should maintain normal standards of good laboratory practice.

5.2.3. If not stated, tolerances required for the various measurements used are:

Temperature $\pm$ 1°C; Time $\pm$ 1 minute; Volumes & Weights $\pm$ 1%

5.2.4. Because of the extreme sensitivity of the test, very high standards of cleanliness should be observed when handling Laboratory Samples/Test Portions, using equipment and cleaning down before, between and after all stages in the process. Homogeniser tools are difficult to clean properly therefore the shake/heat method is preferable to reduce cross-contamination. Use of a swabbing kit (e.g. **A6008 - 100 swabs /A6009 – 25 swabs**) can help validate manufacturing equipment/laboratory cleaning regimes.

5.2.5. Proteins bind strongly to some plastics e.g. polystyrene; it is recommended that extraction & dilution tubes are disposable polypropylene or glass.

5.2.6. To prevent cross-contamination, pipette tips should not be reused.

5.2.7. Ethanol extracts can be difficult to pipette due to pressure build up in the tip. “Reverse” pipetting is preferred for air displacement pipettes; rinse tip several times before pipetting out. If possible use a positive displacement pipette e.g. Gilson Microman. Avoid drops of reagent on the outside of the tip entering wells by wiping carefully with a clean tissue.

5.2.8. The key to good results is consistency from sample to sample & well to well; work quickly but carefully to avoid assay drift; small (32/48 well) assays are preferred. Duplicate wells (at least some!) are strongly recommended.

5.2.9. If required for re-testing, Laboratory Sample/Control extracts can be stored ***at ROOM TEMPERATURE***; they remain stable for several weeks. If refrigerated, warm to re-dissolve extraction solution components then mix well & centrifuge/settle before testing.

5.2.10. If you require extra Diluent/Wash Solution concentrate e.g. if performing a large number of high dilutions of extracts, or using an automated ELISA instrument, please contact Bio-Check (UK) or your local distributor for additional supplies.

6. **KIT CONTENTS PROVIDED:**

Contents vary slightly depending on kit size i.e. 48-, 96- or 192-well presentations – see below.

6.1. **Kit Controls**

6.1.1. Low gluten breadcrumb preparations at two levels:

6.1.1.1. **One x LOW Control (<1 mg/kg)**; breadcrumbs.

6.1.1.2. **One x MID Control blue** breadcrumbs (for mg/kg values ***refer to CofA***).

6.1.1.3. 6g of each per pot; 1 of each; ready to be extracted.

6.1.2. **Diluent Concentrate 1 (5X Concentrate)**.

6.1.2.1. 1 x or 2 x 25mL; to be diluted with purified water.

6.1.3. **Gluten Standards (ppm values below are the "As Diluted" concentrations)**.

6.1.3.1. 1.0mL each of five **20X CONCENTRATE** Standards, with red dye. The five Standards have a gradation of red colour from 5PPM-110PPM as a pipetting aid.

6.1.3.1.1. Gluten **5 PPM; mg/kg** Standard. **BLUE** code.

6.1.3.1.2. Gluten **10 PPM; mg/kg** Standard. **GREEN** code.

6.1.3.1.3. Gluten **25 PPM; mg/kg** Standard. **YELLOW** code.

6.1.3.1.4. Gluten **50 PPM; mg/kg** Standard. **ORANGE** code.

6.1.3.1.5. Gluten **110 PPM; mg/kg** Standard. **RED** code.

6.1.3.1.6. To be diluted with 1X Diluent – see 9.2 on page 9.

6.1.3.2. **IMPORTANT NOTE: WARM thoroughly to room temperature and mix VIGOROUSLY to ensure that the gliadin is fully solubilised.**

6.1.4. **Anti-Gliadin Microwell Plates** (1 x 48, 1 x or 2 x 96 wells).

6.1.4.1. Foil sealed in a re-sealable pouch with a harmless desiccant (yellow or yellow/green if still active; green if exhausted).

6.1.5. **Wash Solution Concentrate (20X Concentrate)**.

6.1.5.1. 1 x, 2 x or 4 x 25mL; to be diluted with purified water.

6.1.6. **Anti-Gliadin HRP** reagent.

6.1.6.1. 1 x or 2 x 12mL or 1 x 24mL; ready to use; contains green food dye as pipetting aid.

6.1.7. **TMB Substrate**

6.1.7.1. 1 x or 2 x 12mL or 1 x 24mL; ready to use. CARE: LIGHT SENSITIVE (turns blue!)

6.1.8. **Acid Stop Solution**

6.1.8.1. 1 x 24mL of 0.5M H_2SO_4.

6.1.9. Laboratory **Sample Preparation & Assay Layout Guide** forms.

6.1.9.1. Photocopy as required; write in assay layout & use as a pipetting guide.

6.1.10. **Gluten Extraction Kit** (included with R6005-1E; R6006-2E & R6007-4E Cat. Nos.)

6.1.10.1. 1 x, 2 x or 4 x boxes containing 24 pre-dispensed tubes of 3.5mL of gluten extraction solution (buffered ethanolic solution with a tannin binding component, yellow dye as a pipetting aid and preservative) and 24 spoons/scoops.

7. EQUIPMENT & MATERIALS

7.1. WHAT YOU NEED (NOT INCLUDED):

7.2. Sample mill, chopper or blender for Laboratory Sample preparation.

7.3. Water bath; set at 55°C.

7.4. 2-Place balance.

7.5. Purified water for Extraction, 1X Diluent & 1X Wash solution preparation.

7.6. Ethanol (absolute); fish skin gelatin (Sigma No. G7765) and Polyvinyl-pyrrolidone (PVP 10; Sigma No. PVP 10) to prepare Extraction Solution.

7.7. ALTERNATIVELY: use our Gluten-Check Extraction Kit (Cat. No. A6017; 24 ready to use tubes each containing 3.5ml of extraction solution with yellow dye as a pipetting aid). E*xtraction kits are included with* **Cat. No. R6005-1E/R6006-2E/R6007-4E** *kits; no need to source extraction reagents; reduces hands-on time; eliminates gluten contamination during preparation.*

7.8. Containers for making up and containing extraction, diluent, wash solutions.

7.9. Polypropylene or glass containers for test portion extraction (~7-20mL; not required if using A6017), centrifugation (if possible use extraction tube) and dilution (~1.5-5mL).

7.10. Microlitre pipettes and tips (e.g. for 100µL & 1,900µL volumes).

7.11. Wash bottle with fine spout and absorbent paper towel for microwell washing.

7.12. ELISA plate reader with 450nm wavelength filter.

7.13. OPTIONAL MATERIALS/EQUIPMENT:

7.14. Centrifuge capable of achieving at least 500g; 1000g – 2000g is preferable. The use of a centrifuge capable of directly spinning extraction tubes saves time and reduces the possibility of cross-contamination between extracts.

7.15. The use of a (100µL) repeating pipette, dispensing syringes and tips helps speed the addition of HRP, TMB & Stop reagents, minimising assay drift.

7.16. The use of an automated or hand-held ELISA plate washer system reduces the time taken to wash plates and can improve consistency.

7.17. ELISA software greatly reduces the time required to calculate results.

8. PREPARATION OF THE EXTRACTION SOLUTION & KIT REAGENTS

8.1. If not using ready-prepared **Extraction Solution** (Cat. No. A6017; 24 sets), prepare gluten extraction/tannin binding solution (approx. 4mL needed for each Sample). For **TEN Samples**:

8.1.1. Add 16ml (12.6g) ethanol, 2.0g fish gelatin and 0.8g PVP to ~20ml purified water and mix well. When dissolved (gentle warming helps dissolve PVP10) make up to 40mL.

8.1.1.1. Stable for at least 2-3 weeks; store at room temperature; MIX WELL prior to use.

8.1.1.2. Note: Specific Gravity ~0.96g/mL.

8.1.1.3. Add 3.5mL (3.36g) of solution to one tube for each Laboratory Sample.

NOTE: If it proves difficult to make the Sample homogeneous, it may be advisable to extract more sample - e.g. add 1.0g to 9.0mL of Extraction Solution - to improve the accuracy of your determination.

8.2. Prepare **Diluent Solution 1 (1X)**.

8.2.1. Note: this concentrate can form crystals if stored refrigerated. Help avoid this by removing from kit and storing at Room Temperature, or warm prior to use to re-dissolve any crystals which may have formed.

8.2.2. Dilute Diluent Concentrate 1 (5X) at a ratio of 1:4 (1/5) in purified water, or add bottle contents to 100mL of water.

8.2.3. The 1X Diluent is used to dilute Standards and Control & Sample extracts and as a **ZERO Standard** in the assay. Standards, Control/Sample extracts MUST all be diluted 1/20 in 1x Diluent before being assayed – see 9.2.

8.3. Prepare **Standards.**

8.3.1. **WARM the 20X Concentrate Standards thoroughly to room temperature.**

8.3.2. **Mix the Standards VIGOROUSLY so that the Standard solutions are clear and to ensure the gliadin is fully solubilised prior to dilution – see 9.2 for dilution details.**

8.4. Prepare **Wash Solution (1X).**

8.4.1. Note: this concentrate can form crystals if stored refrigerated. Help avoid this by removing from kit and storing at Room Temperature, or warm prior to use to re-dissolve any crystals which may have formed.

8.4.2. Dilute Wash Solution Concentrate (20X) at a ratio of 1:19 (1/20) in purified water, or add bottle contents to 500mL of water.

8.4.3. The 1X Wash Solution is used to wash the microwells during the assay.

8.5. Anti-Gliadin Microwell Plate(s).

8.5.1. Cut or carefully tear foil pouch between the two notches; pull open pouch closure and remove plate. Carefully remove strips not required for the ELISA from the plate frame.

8.5.2. Re-seal remaining wells in pouch by pressing the re-sealable closure across its width; ensure that desiccant is present and active (yellow to pale green/yellow).

9. PREPARATION OF YOUR SAMPLES and the KIT CONTROLS

9.1. SWABBING & LABORATORY SAMPLES/KIT CONTROL FLOURS

9.1.1. Swabbing samples (e.g. A6008/9) can be assayed undiluted – proceed to 11.1.

9.1.2. Finely divided flours/powders (including Kit Controls), fine breadcrumbs and smooth liquids require no preparation – proceed to 9.1.4.

9.1.3. For non-homogeneous Samples, take out a representative portion of the sample and prepare by milling, grinding, chopping, blending etc until it has a fine particle size and/or appears to be homogeneous.

9.1.4. Weigh out one part of Test Portion e.g. 0.35g into 3.5mL (3.36g) of Extraction Solution in e.g. a polypropylene container.

9.1.5. Record exact weight added on Layout Guide. To save time, you do not have to add exactly 0.35g, BUT make sure you record the weight and correct back for the actual weight used when calculating results – see p.12.

9.1.6. Shake vigorously by hand preferably on a mixer (1,000 rpm) for at least 2 minutes to disperse Test Portion in the extraction solution and extract any gluten present.

9.1.6.1. Place samples in water bath at **55°C for 45 MINUTES**.

9.1.6.2. Shake vigorously for a few seconds once during the extraction.

9.1.6.3. At the end of the extraction, shake vigorously (see 9.1.6) for at least 2 minutes.

9.1.7. Either pour a small portion of the extract into a suitable centrifuge tube or, if possible, spin the whole tube. Centrifuge at ≥500g for 10-15 minutes. Alternatively, settle for ~30 minutes or until a liquid extract layer appears above the solid food.

9.1.8. If a fatty layer appears above the extraction liquid it is best to remove it by e.g. careful aspiration with a vacuum line. Alternatively, remove a portion of extract liquid using a clean Pasteur pipette and place in a clean polypropylene tube.

9.2. Prepare **1/20 dilutions** of the well-mixed **20X Concentrated Standards** and the **Sample Extract** liquids:

- e.g. carefully pipette out e.g. a **100µL** (0.100mL) portion of extract from the liquid layer above the food pellet and add to **1,900µL** (1.900mL) of 1X Diluent Solution 1. Mix well.
- If using extraction kit A6017 the dye present allows instant recognition of those Samples/Controls that have been diluted, which should be pale yellow in colour.

NOTES: A 1/20 dilution is preferred for Samples described as “gluten free” or “very low gluten” (standard curve equates to **5 to 110 PPM** gluten).

For foods expected to contain higher levels of gluten, make an additional dilution of the extract e.g. 1/2, 1/5, 1/10 or 1/100 with Diluent 1 (1X), depending on how high it may be, then dilute as per 4.1.9 above.

- If diluting an additional 10-fold the standard curve equates to 50 to 1,500 PPM.
- If diluting an additional 100-fold the standard curve equates to 500 to 15,000 PPM gluten).

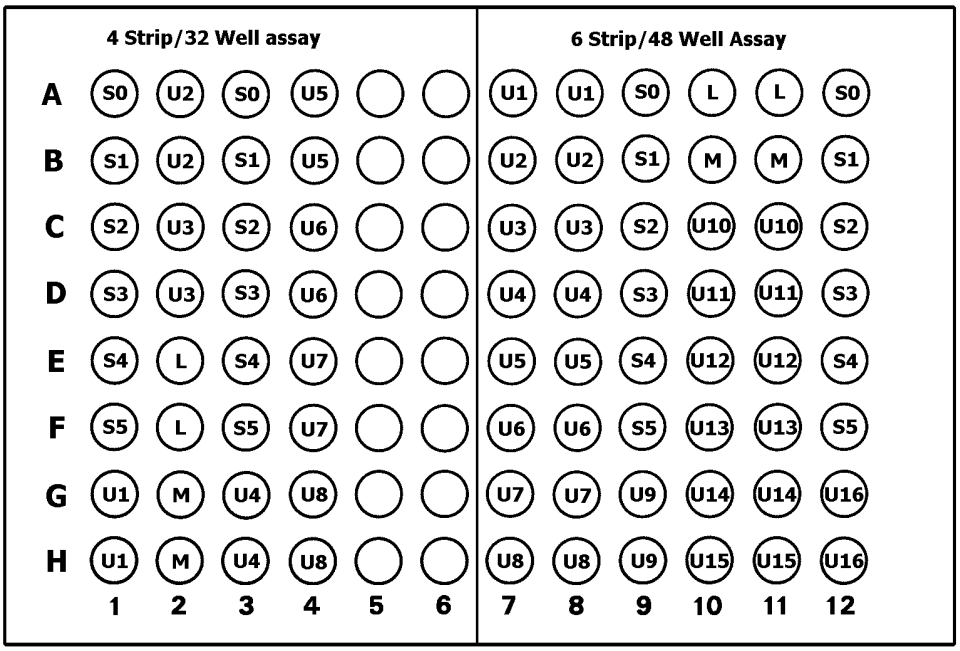
To reduce the assay range (to 2.5 to 75 PPM gluten), dilute extracts 1/10 e.g. add 100µL (0.100mL) to 900µL (0.900mL) of 1X Diluent.

Swabbing solutions can be further diluted in Diluent 1 (1X) if required to aid quantitation.

9.3. The **diluted Standards/extracts** are now ready for ELISA testing (section 11; page 11).

10. **EXAMPLE ASSAY LAYOUT**

10.1. Suggested Assay Layouts (32 & 48 well assays).



Key to Layout:

S0 – S5

Gluten Standards

(Zero-110PPM)

L , M

Kit Controls

U1 – U16

Sample Extracts

11. DETAILED ELISA PROCEDURE

11.1. Allow all kit reagents to reach room temperature (18-24°C preferable); prepare Test Portion & Control extracts, 1X Diluent & Wash Solutions, Standards & Sample Extract dilutions, all as described above.

11.2. Ensure that the work area is well organised and tidy, all dilutions are clearly labelled in the correct order (Layout Guide) for pipetting and that ELISA equipment is ready for use; remove caps from all dilutions to speed up addition to the wells.

11.3. Mark microwell strips on upper or lower tab (or individual break-apart wells on side) to keep them in the correct order should they become detached from frame.

11.4. Mix ready to use HRP, TMB and Stop reagents gently just before use.

11.5. Quickly but carefully "reverse" pipette, according to the Layout Guide you set out, 100µL of: Zero (S0; 1X Diluent) & S1–S5 dilute Standards, diluted Low & Mid Kit Control extracts & diluted Sample Extracts into appropriate wells using a microlitre pipette.

11.6. Mix the plate by sliding back and forth, gently but briskly, in short movements (1-2cm side to side) on a smooth surface.

11.7. Cover the plate and incubate at room temperature for **20 MINUTES**.

11.8. **WASH WELLS THREE TIMES**: Empty wells by flicking out contents into a sink; carefully fill each well in turn using a wash bottle containing 1X Wash Solution. Repeat emptying and filling cycle twice more. After the three wash cycles, flick out the plate several times to remove excess liquid; tap the wells upside down FIRMLY on absorbent paper until little or no liquid appears on the paper; while inverted, wipe base of wells to clean them.

Alternatively: Use a hand held/automatic plate washer to aspirate then fill wells three times; empty wells then tap onto paper as described above to remove excess liquid.

11.9. Immediately add 100µL of Anti Gliadin HRP (green coloured reagent) using a microlitre or repeating pipette; mix as described in 11.6.

11.10. Cover the plate and incubate at room temperature for **20 MINUTES**.

11.11. Wash all wells **FOUR** times with 1X Wash Solution as in 11.8.

11.12. Immediately add 100µL of TMB Substrate to all wells; mix as described in 11.6.

11.13. Cover plate; incubate at room temperature **IN THE DARK** (e.g. in a drawer) for **20 MINUTES** or until sufficient colour develops (sections 15/16; p. 14/15).

11.14. Add 100µL of Stop Solution 1 to all wells (blue to yellow colour change in wells).

11.15. Mix plate as described in 11.6 to stop enzyme activity and evenly distribute colour. (Colour stable for at least 60 minutes).

11.16. Read plate at 450nm using the plate reader and record absorbance values.

12. **CALCULATION OF RESULTS**

12.1. Plot a Standard Curve of OD450nm values against Standard mg/kg (PPM) GLUTEN values on normal/semi-log graph paper; draw a curve/line of best fit.

12.1.1. Read off Kit Control & unknown Sample gluten concentrations (PPM; mg/kg) off the Standard Curve; record results on Layout Guide provided if appropriate.

12.2. Alternatively use curve-fit software (4-Parameter Logistic fit preferred) to produce the results.

12.3. **"Non-Standard" Calculations**: if appropriate (i.e. if the nominal 1:10 Extraction and 1/20 Dilution ratios are NOT used) correct the PPM (mg/kg) result by adjusting for the actual weight/volume of sample/extraction solution used in the extraction step and by the actual extract dilution used:

For example if 0.327g sample was extracted instead of 0.350g and a 1/40 dilution used instead of 1/20, a value of 44 PPM (mg/kg) Gluten read of the Standard Curve is equivalent to:

$$\frac{44 \times 0.350 \times 40}{0.327 \times 20} = \frac{616}{6.54} = \textbf{94.2 PPM Gluten content}$$

12.4. **Gliadin to Gluten calculations**: the Standards' **Gliadin** concentrations are:

12.5, 25, 62.5, 125 & 275 ng/mL.

12.4.1. **A factor of 2.0** is used to convert from gliadin to gluten levels (It is generally agreed that gluten contains ~50% gliadin therefore: 2 x gliadin = gluten).

12.4.2. Gliadin values are further corrected **by dividing by 1,000** to convert from ng/g to mg/kg (PPM) gluten.

For example, if a Sample is extracted and diluted exactly as described in the IFU and returns a value of 220ng/mL **gliadin**, its PPM/mg/kg **gluten content** is:

$$\frac{\mathbf{220} \times \mathbf{V} \times \mathbf{D} \times 2}{\mathbf{W} \times 1000} = \frac{220 \times 3.5^{\dagger} \times 20^{\dagger} \times 2}{0.350^{\dagger} \times 1 \times 1000} \equiv \frac{30{,}800}{350} = \textbf{88.0 PPM Gluten}$$

† Substitute actual values of **V** (Volume of Extraction Solution), **D (**Dilution ratio used) & **W** (Weight of sample extracted) for each Control/unknown Sample.

If a Sample is extracted and diluted as per 12.3 above, and returns e.g. a value of 220ng/mL **gliadin**, its PPM (mg/kg) **gluten content** is:

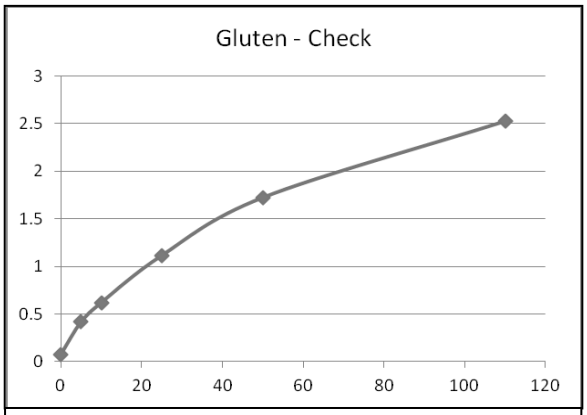
$$\frac{\mathbf{220} \times V \times D \times 2}{W \times 1000} = \frac{220 \times 3.5^{\dagger} \times \mathbf{40}^{\dagger} \times 2}{\mathbf{0.327}^{\dagger} \times 1000} \equiv \frac{61{,}600}{327} = \textbf{188 PPM Gluten}$$

12.5. If testing **swabbing samples**, (e.g. collected using our ESS Environmental/Surface Swabbing kits, Cat. No. A6008 [100 sets] and A6009 [25 sets]) the PPM gluten value for the swabbing solution read off the standard curve must be divided by e.g. 200 to adjust for the fact that the swab solution is neither extracted (normally 1:10 ratio) nor diluted (normally 1/20 ratio). If appropriate, you may wish to correct for approximate recovery from the swabbed surface.

13. **EXAMPLE ASSAY DATA** Bio-Check Gluten-Check ELISA (Assay ID: 120828.1P)

13.1.	Zero Standard:	OD_{450}	0.074
13.2.	5 PPM	OD_{450}	0.416
13.3.	10 PPM	OD_{450}	0.619
13.4.	25 PPM	OD_{450}	1.115
13.5.	50 PPM	OD_{450}	1.723
13.6.	110 PPM	OD_{450}	2.525
13.7.	Kit Control (Low)	OD_{450}	0.075
13.8.	Kit Control (Low)	PPM	0
13.9.	Kit Control (Mid)	OD_{450}	1.204
13.10.	Kit Control (Mid)	PPM	28.0

13.11. Example Bio-Check Gluten-Check ELISA Standard Curve (right**).**



Standard OD_{450nm} readings (Y) against PPM (mg/kg) GLUTEN

14. INTERPRETATION OF RESULTS

14.1. Assay Calibration. At present there is no agreed calibrator to help support gluten analysis. The Gluten-Check ELISA has been calibrated using retail, plain white wheat flour (10.2% protein), "Consensus Values" for samples previously characterised in the FAPAS Proficiency Testing Scheme (www.fapas.com) and our own in-house Assayed Q.C. panel of "gluten free"/"very low gluten" and high gluten level food samples.

14.2. Currently a maximum level of 20PPM is indicated by Codex 118 for foods labelled as "Gluten Free" and >20<100PPM for foods to be labelled "very low gluten" and designed for PARNUTS use to be consumed by those with Coeliac Disease or wheat intolerant individuals.

14.3. Cereal reactivity: the antibody used in this kit reacts to:

✓ Rye/Triticale at a level of	~115%	}	
✓ Durum wheat	~50%	}	all relative to wheat = 100%.
✓ Barley, the least toxic cereal	~21%	}	

The antibody also responds very strongly to other wheat-related species (kamut; spelt) and to barley malt preparations.

14.4. CROSS REACTIVITY: The following pure commodities DO NOT react in the assay:

Almonds	Amaranth	Brazil nut	Buckwheat	Cashew
Chickpea	Cornflour	Hazelnut	Lentil (green)	Lentil (red)
Lupin flour	Maize flour	Milk/whey	Milk/casein	Millet
Oats	Pecan nut	Pine nuts	Pistachio	Potato/potato starch
Pumpkin seed	Quinoa	Rice flour	Sesame	Soya flour
Sugar	Sunflower seed	Chocolate (milk or plain)		

15. PERFORMANCE INDICATIONS

15.1. Prior to stopping the ELISA, S0 wells should be nearly colourless and there should be a definite colour difference between the S0 and pale blue S1 (5PPM) wells. The S5 (110PPM) wells should be a mid blue colour. Indicative assay parameters are suggested to be as follows:

15.2.	Zero OD_{450}nm:	<0.15 units
15.3.	Limit of Detection:	<1 PPM (at 3 x Std. Dev. from Zero)
15.4.	5 PPM OD_{450nm}:	>3.0 x Zero OD450nm
15.5.	110 PPM OD_{450nm}:	>1.25 units; preferably >1.75 units.
15.6.	Kit Control (LOW / MID):	LOW: < 5 mg/kg; MID: Refer to C of A
15.7.	Duplicate precision (OD_{450nm}):	Ideally <5%
15.8.	Duplicate precision (PPM gluten):	Ideally <10 – 15%

In our laboratories the average Limit Of Detection and % Coefficient of Variation of gluten concentration replicates are routinely <0.25 PPM and <7.5% respectively.

16. PROBLEM SOLVING

16.1. Regular maintenance and calibration of equipment, especially microlitre pipettes, helps improve assay performance.

16.2. Good laboratory practice reduces the possibility of cross contamination; swabbing kits (e.g. our Cat. Nos. A6008 & A6009; 100 & 25 swabbing sets respectively) can help validate and verify laboratory/equipment cleaning regimes.

16.3. Use of the Gluten-Check Extraction Kit (Cat. No. A6017; 24 ready to use tubes each containing 3.5ml of extraction solution with yellow dye as a pipetting aid) removes the need to source extraction reagents, reduces hands-on time significantly and eliminates contamination of the extraction solution with gluten during preparation.

16.4. Poor replication is most often due to poorly maintained pipettes or inadequate/inconsistent plate washing.

16.5. Pipettes: ensure that all pipettes are kept in good condition and are regularly calibrated.

16.6. Try to avoid bubbles in the wells during the last wash by carefully overfilling, especially when using a wash bottle. If using a hand-held washer the bubbles can be aspirated away; if using a wash bottle, flick out well contents vigorously. After washing, tap **vigorously** on absorbent paper towel until no bubbles remain in the wells and little or no liquid appears on the paper towel.

16.7. Consistently attaining ideal levels of colour development (see example data section 13; page 12) depends on:

16.7.1. laboratory temperature; at temperatures below 18°C incubation times tend to be longer and above 22°C they may need to be shortened.

16.7.2. effectiveness of washing; ensure that wells are filled to the rim and remember, it is difficult to over-wash!

16.7.3. plate reader range; some readers can measure up to an absorbance as high as 3 units or more, whereas others are limited to <2 units. It is important to judge colour development to fit the range of your reader.

16.7.4. previous experience of this ELISA in your lab.

16.7.5. If you also have a 620nm filter you can monitor colour development after ~nine minutes to help predict final (stopped) OD450nm values. These will be ~3 times the predicted OD620nm level at ten minutes. Stop the assay when the OD620 value is expected to be between 0.5–0.8 units.

16.8. If your plate reader has a pre-mixing facility, set the speed to between 700-900 cycles per minute and time for ~10 seconds.

17. RECYCLING

Wherever possible, we recycle our waste materials. Please help our environment by recycling the paper/card, plastic & glass used in this kit and during the extraction & dilution processes.

Remember the recycling mantra:

- ✓ Reduce
- ✓ Reuse (with care – avoid cross contamination!)
- ✓ Recycle

Bio-Check (UK) Limited ensures that its products are made from high quality raw materials but can make no warranty, express or implied, as to their suitability other than to measure gluten content when used exactly in accordance with these instructions.

Use of the kit for any other purpose is outside its intended use.

Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

The following food testing products are also available in the range:

Gluten Extraction kits – save time and money.

Swabbing Kits – for environmental surfaces in factories/laboratories.

Assayed Q.C. Controls (Negative & Positive BREAD or FLOUR)

GLUTEN FlowThrough™ Tests: for gluten testing in small laboratories or in food manufacturing plants without laboratory facilities for e.g. raw materials, finished products, environmental swabs & finished product testing (detects 10-20PPM gluten in food; <1PPM in swabs).

Allergen-Check ELISA kits for: Almond; Crustacea; Egg; Egg (in wine); Fish; Hazelnut; Lupin; Milk (Casein); Milk (B-LG); Milk (Total); Mustard; Peanut; Pistachio; Sesame; Soya; Walnut

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