BioFront MonoTrace™ Gluten ELISA Kit

Store contents at 2-8˚C

A Monoclonal Antibody-Based Enzyme-Linked Immunosorbent Assay (ELISA) for the Quantitative and/or Qualitative Detection of Gluten

Read instructions carefully before using kit

TABLE OF CONTENTS

Page

DESCRIPTION AND INTENDED USE 1

SPECIFICATIONS 2

REQUIRED MATERIALS 2-3

KIT CONTENTS 2
RECOMMENDED EQUIPMENT 3

ASSAY PROCEDURE 3-5

IMPORTANT NOTES 3
PREPARATION OF REAGENTS 4
PREPARATION OF SAMPLES 4
SAMPLE EXTRACTION 4
RECOMMENDED ELISA PROCEDURE 5

ANALYSIS OF RESULTS 5

QUALITATIVE ANALYSIS 5
QUANTITATIVE ANALYSIS 5
PERFORMANCE INDICATIONS 5

ASSAY CLAIMS 6

SHELF LIFE 6

MATERIAL SAFETY DATA SHEET INFORMATION 6

WARRANTY 6

REFERENCES 7

CUSTOMER SERVICE 7

DESCRIPTION AND INTENDED USE

The BioFront Technologies MonoTrace™ Gluten ELISA kit is a novel sandwich enzyme-linked immunosorbent assay (ELISA) for the quantitative or qualitative detection of gluten in food samples. The kit uses a novel pair of highly sensitive monoclonal antibodies to specifically target and detect the α-gliadin component of gluten. The MonoTrace™ Gluten ELISA kit incorporates a new single-step non-toxic extraction method (patent pending) resulting in a highly sensitive and specific method for the detection of gluten in raw ingredients and processed foods. The United States Food and Drug Administration (FDA) and Codex Alimentarius states that foods declared as gluten-free must contain less than 20 parts per million (ppm) gluten.

SPECIFICATIONS

Testing Time (post-extraction):	**~30 minutes**
Number of Test Wells per Kit:	**48 (GLU-EK-48) or 96 (GLU-EK-96)**
***Limit of Detection:**	**0.36 ppm gluten (0.18 ppm gliadin)**
****Range of Quantification:**	**2 to 100 ppm gluten (1 to 50 ppm gliadin)**
Specificity:	**Prolamins from wheat (gliadin), rye (secalin) and barley (hordein)**
Cross-Reactivity	The assay exhibits strong reactivity to wheat, barley, and rye flours. Millet flour (0.0012%) and corn flour (0.0004%) were minimally cross-reactive in the assay. **No additional cross-reactivity** was observed in a large panel of assayed samples, including: **cereals, tree nuts, legumes, seeds, meats and spices.**
Recovery	Recovery of gluten spiked at various concentrations was within acceptable limits according to current AOAC guidelines[1] when a broad range of matrices considered to be important foods for individuals following a gluten-free diet were assayed.[2]

For more details on assay parameters, a full validation report is available upon request.

Note: calculations of parameters are based on representative data from multiple assays using 10-minute incubation steps at room temperature (20-23.5°C / 68-74.3°F). Higher temperatures may result in elevated absorbance readings for samples and standards.

*The limit of detection (LOD) was determined statistically based on the standard deviation (SD) of the response and assay background according to the formula: LOD = background + 3X SD.

**The range of quantitation (ROQ) was determined experimentally, whereby the lower limit of the ROQ is defined as the lowest concentration at which the assay can reliably and accurately quantify gluten in a sample. For quantitation of samples with gluten above 100 ppm, samples should be diluted such that the results fall within the ROQ (2 to 100 ppm gluten).

REQUIRED MATERIALS

Kit contents

Reagent	Amount within 48-well kit (GLU-EK-48)	Amount within 96-well kit (GLU-EK-96)
Assay plate, one 96-well plate	Six 8-well strips *Sufficient for 48 or 96 assay values, including standards & blank*	Twelve 8-well strips
10X MGEB = 10X MonoTrace gluten extraction buffer	50 mL *Sufficient to extract 12 x 1.0 g or 1.0 mL samples; for additional extraction buffer, please contact one of our representatives*	
5X SD = 5X sample diluent	50 mL *Sufficient to dilute samples for > 96 assays wells*	
Ready-to-use gluten calibrators: **100, 40, 20, 5, 2 and 0 ppm**	1 mL of each standard *Sufficient for 10 standard curves*	2 mL of each standard *Sufficient for 20 standard curves*
10X WB = 10X wash buffer	50 mL *Sufficient wash buffer for > 96 wells*	
CON = 1X anti-gluten HRP-conjugate	12 mL *Sufficient for > 48 wells*	15 mL *Sufficient for > 96 wells*
SUB = high sensitivity TMB substrate	12 mL *Sufficient for > 48 wells*	15 mL *Sufficient for > 96 wells*
STOP = HRP quench solution	12 mL *Sufficient for > 48 wells*	15 mL *Sufficient for > 96 wells*

Recommended equipment

- Absolute ethanol (200 proof)
- 1 L graduated cylinder
- Storage bottle for 1X MGEB
- Water bath for 60°C incubations
- Timer
- Balance or scale capable of measuring milligram quantities
- 1.5 or 2.0 mL microfuge tubes and tube racks
- 15 or 50 mL conical tubes
- Distilled water or equivalent
- Pipet-Aid® (or equivalent) and serological pipettes, capable of measuring 5-50 mL
- *****Single and multichannel pipettes, capable of measuring 1-1,000 µL
- *****96-well assay blocks (Corning® product no: 3961 or equivalent)
- *****Reagent reservoirs
- Absorbent paper (to slap dry plates) or ELISA plate washer
- Centrifuge, capable of 2,000 x g
- Vortex
- Microplate reader, capable of reading absorbance at 450 nm

**Note: It is recommended that assay blocks, reagent reservoirs (basins) and a multichannel pipette be used to obtain the most accurate results. For assays in which more than 16 samples will be tested, these accessories are required for the efficient dispensing of reagents to insure minimal variations in incubation times between assay wells.*

GLUTEN ASSAY PROCEDURE

It is important for the user to read all instructions carefully before performing an assay.

Important notes

Extraction

The MonoTrace™ Gluten ELISA assay is extremely sensitive; capable of detecting minute amounts of gluten. Careful consideration should be given to the preparation of test materials to ensure several important parameters:

1. The equipment used to prepare samples must be thoroughly cleaned to prevent the contamination of subsequent samples.
2. Disposable plasticware (tubes, pipette tips, etc.) are used wherever possible.
3. The samples are homogenized completely to prevent excessive intra-sample variation.
4. The supplied extraction buffer is sufficient for up to twelve 1-gram or 1 ml samples. If other quantities are used, a 40:1 buffer/sample ratio should be maintained (40 mL extraction buffer per 1 gram sample). Additional extraction buffer is available for purchase from BioFront Technologies if needed.
5. Food matrices containing high concentrations of solid fats, such as chocolate, may require additional heating to completely melt the sample before adding preheated extraction buffer.
6. Food matrices such as spices, dark chocolate, dairy products, or those containing polyphenols, such as tannins require the addition of 2.5% fish gelatin to the extraction buffer to achieve optimal results. If performance issues with a certain matrix are suspected, please contact a BioFront Technologies representative for guidance.

Spike control preparation (optional)

Some food matrices may alter the recovery and sensitivity of the ELISA. If suspected, unspiked and gluten-spiked control matrices can be tested. Values obtained on test samples can then be adjusted accordingly. Ready-to-use standards provided with the kit are meant to serve only as calibrators for the assay and are NOT to be used as spiking agents. Doing so will lead to erroneous results. For help with setting up your matrix validation through the testing of spiked samples or to request that a unique matrix be validated by BioFront Technologies, please contact one of our representatives.

Preparation of reagents (extraction buffer, sample diluent, and wash buffer)

1. Determine the amounts of reagents needed.
2. Prepare 1X extraction buffer (MGEB) by diluting the concentrated **10X MGEB** in 60% ethanol using absolute ethanol (200 proof) and distilled water or equivalent.
    - To prepare 100 ml of 1X MGEB, add 10 ml of the **10X MGEB,** 30 ml of distilled water and 60 ml of absolute ethanol. Prior to performing sample extraction, preheat the 1X MGEB to 60°C (140°F).
    - To prepare 100 ml of 1X SD, add 20 ml of the **5X SD** to 80 ml of distilled water.
    - To prepare 100 ml of 1X WB, add 10 ml of the **10X WB** to 90 ml of distilled water.
3. All 1X working solutions should be equilibrated to room temperature prior to use. Unused 1X solutions can be stored refrigerated (2-8 °C) for up to 1 month for future use. If buffers appear cloudy after equilibration to room temperature, they may be contaminated and should be discarded.

Preparation of samples

To insure adequate sampling, it is important that a representative amount of sample (at least 5 grams) be thoroughly homogenized and the particle size rendered as small as possible. Blending/grinding to a fine powder/flour is strongly recommended. Small particle size also enhances extraction efficiency.

Solid/liquid sample extraction (read 'important notes' section prior to this step)

1. Transfer 1 gram of the finely ground food sample or 1 mL of liquid sample to a ≥ 40 mL tube.
2. Add 40 mL of preheated 1X extraction buffer (diluted **MGEB)** to the mixture and briefly vortex to suspend the contents. If other starting quantities are used, a 40:1 buffer/sample ratio (40 ml extraction buffer per 1 gram sample) should be maintained.
3. Incubate tubes at 60°C (140°F) for 1 hour, mixing vigorously every ~15 minutes.
4. Spin extraction samples at 2,000 x g for 10 minutes at room temperature and transfer the aqueous phase into fresh tubes for testing.

Recommended ELISA procedure

1. Determine the number of assay wells needed for test samples and standards. Carefully remove the strips that are **not** to be used by gently pushing them from beneath the plate until they pop out and return them to the Mylar™ bag. Seal tightly and store at 2-8°C.
2. Dilute sample extracts 10-fold in 1X sample diluent (1 volume of extracted sample to 9 volumes diluted **SD**). For instance, add 100 µL of extract into 900 µL of sample diluent.
3. Add 100 µL of diluted samples and ready-to-use standards to the appropriate wells.
4. Incubate plate at room temperature for 10 minutes.
5. Discard well contents, blot onto absorbent paper with a slapping action (or autowash). Wash 3X with 1X wash buffer, (diluted **WB**) using ≥ 200 µL per well per wash, and blot dry.

6. Add 100 µL of 1X anti-gluten-conjugate (diluted **CON**) to each well.

7. Place plate in dark environment and incubate at room temperature for 10 minutes.

8. Discard well contents, wash, and blot dry as described in step #5.

9. Add 100 µL of HRP substrate (**SUB**) per well.

10. Incubate plate in dark for 10 minutes.

11. Add 100 µL of quench solution (**STOP**) to each well and mix by gently pipetting so as to prevent bubbles that could interfere with absorbance readings.

12. Read the absorbance of the wells using a plate reader programmed with a primary absorbance filter of 450 nm and a differential filter of 630 nm. For some plate readers, the differential filter may be automatically accounted for and reading only at 450 nm will be required. Please consult your reader user manual for more information.

13. Plot the standard curve. Interpolate unknown data using the standard curve and appropriate dilution factor. If the recommended ELISA procedure above is followed, no dilution factor is required as the initial 10-fold dilution is accounted for by the assay. Should, however, further dilution be required to obtain results within the assay's ROQ, then the additional dilution factor must be taken into consideration when sample concentrations are determined. BioFront Technologies offers users a MonoTrace ELISA calculation template in Excel format to help simplify sample calculations.

ANALYSIS OF RESULTS

Qualitative analysis of assay results

A qualitative assessment can be made using one or more of the provided assay standards. Any of these standards can be used to define a specific threshold to which the unknown sample can be compared. Samples with absorbance values at or above the threshold are considered to be positive, whereas those samples below the threshold are considered to be negative.

Quantitative analysis of assay results

A **standard curve** should be generated from the averaged ODs of the 0-100 ppm standards after subtracting the 0 ppm averaged background values. A third order polynomial (cubic) curve fit is recommended for this evaluation. The ppm concentrations of test samples can be determined by plotting OD values onto the curve and multiplying the calculated concentrations by the appropriate dilution factors (if used). Note that the ppm designations on the provided standards are intended to allow the direct calculated ppm of total gluten in an original food sample, i.e. no dilution factor is applied when calculating results. BioFront Technologies also provides users with a MonoTrace ELISA calculation template in Excel™ format, which can be used to simplify sample quantitation. Please contact a BioFront Technologies representative for more information.

Performance indications

The ready-to-use standards used in the assay should yield OD values in line with those indicated on the accompanying lot-specific quality control document. Significant deterioration in signal may indicate expiration of the reagents. If quantitation is required and the OD of the test sample is above that of the 100 ppm gluten standard, further dilution of the sample should be performed prior to repeating the assay to ensure results fall within the assay's ROQ.

ASSAY CLAIMS

When performed as instructed, the assay is capable of a simple yes/no qualitative assessment of gluten presence in food samples or a quantitative determination of gluten content. Extracted food samples that generate a colorimetric readout can be compared to the linear portion of a standard curve, allowing the interpolation of gluten in ppm. The assay is capable of quantifying gluten content between 2 and 100 ppm.

A negative result by this or any other immunological assay does not assure the complete absence of gluten within the sample. The sample may contain gluten below the limit of detection of this assay or the sample may not be adequately homogenized. The MonoTrace Gluten ELISA kit **does not claim** that food is safe for consumption based upon a determination of gluten content.

SHELF LIFE

Each plate is packed in a vacuum-sealed Mylar™ pouch with a desiccant packet to extend the shelf life of the product to a minimum of six (6) months from the date of manufacture, if stored at 4°C. The stability of the ready-to-use standards may slightly deteriorate over time, as indicated in the certificate of analysis accompanying each kit. BioFront Technologies is happy to provide fresh standards at the customer's request. The performance of the plates can be adversely affected by excessive exposure to light, moisture, and air. It is recommended that the foil pouch and contents be brought to room temperature before removing the contents to avoid condensation.

MSDS INFORMATION

Material safety data sheets are available on the BioFront website, www.biofronttech.com.

WARRANTY

These products are warranted to perform as described herein. All returns must be pre-approved for refund or credit by a BioFront technical representative and are subject to inspection and verification of contents. Failure to comply may result in a delayed or voided refund.

REFERENCES:

1. Abbott M, Hayward S, Ross W, Godefroy SB, Ulberth F, Van Hengel AJ, Roberts J, Akiyama H, Popping B, Yeung JM, Wehling P, Taylor SL, Poms RE, Delahaut P. Validation procedures for quantitative food allergen ELISA methods: community guidance and best practices. J AOAC Int. 2010 Mar-Apr;93(2):442-50.

2. Koerner TB, Abbott M, Godefroy SB, Popping B, Yeung JM, Diaz-Amigo C, Roberts J, Taylor SL, Baumert JL, Ulberth F, Wehling P, Koehler P. Validation procedures for quantitative gluten ELISA methods: AOAC allergen community guidance and best practices. J AOAC Int. 2013 Sep-Oct;96(5):1033-40.

CUSTOMER SERVICE

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