BioFront MonoTrace™ Crustacea 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 Crustacea in Food

Read instructions carefully before using kit

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DESCRIPTION AND INTENDED USE

The BioFront Technologies Crustacea ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative or qualitative detection of Crustacea. This monoclonal antibody (MAb)-based assay provides a highly sensitive and specific method for the quantification of Crustacea presence/contamination within a variety of food matrices. It may also be used for simple “yes-no” qualitative assessments. The kit enables a facile and quantitative measure of Crustacea without the false positive signals often associated with polyclonal antibody (PAb)-based ELISA kits. The target indicator protein is the major Crustacea allergen tropomyosin, which was selected based on its strong resistance to food processing and high abundance.

SPECIFICATIONS

Testing Time (post-extraction): **~30 minutes**

Number of Test Wells per Kit: **96**

***Limit of Detection:** **70 ppb Crustacea**

****Range of Quantification:** **1 to 40 ppm**

Specificity: **Crustacea (see below)**

Crustacea	Processing	Cross-reactivity	Conversion factor [1]
Shrimp	Raw	98%	1.0
	Cooked	59%	1.7
Prawn	Raw	133%	0.8
	Cooked	67%	1.5
Northern lobster	Raw	87%	1.0
	Cooked	60%	1.7
Spiny lobster	Raw	35%	2.8
	Cooked	26%	3.9
Crayfish	Blanched	65%	1.5
	Cooked	120%	0.8
Crab	Raw	63%	1.6
	Cooked	43%	2.3

Cross-Reactivity

The assay exhibits strong reactivity to Crustacea and to a significantly lesser extent octopus (0.0205%).

At concentrations as high as 100,000 ppm mussels (0.0013%) demonstrated minimal cross-reactivity while no such cross-reactivity was observed within a large panel of tree nuts, legumes, vegetables, seeds, flours or meats.

Reactivity towards raw and cooked shrimp, prawn, lobster, crayfish and crab was similar (see above).

Recovery

Recovery of spiked samples was acceptable according current AOAC guidelines (J. AOAC Int. 2012) when the following food matrices were assayed:

soup mix, fish, meat, juices, sauces, condiments, wine, batter mixes, oils and dressings

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 of the response (SD) 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 Crustacea in a sample. For quantitation above 40 ppm, samples should be diluted such that the results fall within the ROQ (1 to 40 ppm).

[1] Conversion factor = an optional value that can be multiplied by sample ppm to generate specific Crustacea signals.

REQUIRED MATERIALS

Kit contents

Reagent	Amount	Use
Assay plate, one 96-well plate	Twelve 8-well strips	Sufficient to obtain 96 assay values, including necessary standards and blank
10X ExB = 10X extraction buffer	50 mL	Sufficient to extract > 96 100 mg samples
5X SD = 5X sample diluent	50 mL	Sufficient to dilute samples and standards for > 96 assays
Ready-to-use Crustacea standards	2 mL each of 40, 20, 10, 4, 1 and 0 ppm	Sufficient for up to ten standard curves
10X WB = 10X wash buffer	50 mL	Sufficient wash buffer for > 96 wells
CON = 1X anti-Crustacea HRP-conjugate	20 mL	Sufficient conjugate for > 96 wells
SUB = high sensitivity TMB substrate	20 mL	Sufficient for > 96 assays
STOP = HRP quench solution	20 mL	Sufficient for > 96 assays

Recommended equipment

- Chamber or waterbath for 60°C incubation
- 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
- *****Reagent reservoirs
- Absorbent paper 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 (boats) and a multichannel pipette be used in order to obtain the most accurate results. For assays in which more than 16 samples will be tested, this is required to insure the incubation time of all samples is universal.*

CRUSTACEA ELISA ASSAY PROCEDURE

It is important for the user to read all instructions carefully before performing the assay. Reagents, including the assay plate, should be equilibrated to room temperature prior to use.

Important notes

Extraction

The Crustacea ELISA assay is extremely sensitive, capable of detecting minute amounts of shellfish. Careful consideration should be given for the preparation of food matrices to ensure 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 fifty 1 gram samples. If other quantities are used, a 10:1 buffer/sample ratio should be maintained (9 volumes of extraction buffer to 1 part sample).
5. Food matrices containing high concentrations of solid fats may require additional heating to completely melt the sample before adding preheated extraction buffer.
6. For spices or food matrices containing polyphenols, such as tannins, found in chocolate, fruits, wine, tea and coffee, the addition of 5% non-fat dry milk to the extraction buffer is recommended to achieve optimal results. If performance issues with a certain matrix are suspected, please contact a BioFront Technologies representative.

Spike control preparation (optional)

Some food matrices may alter the recovery and sensitivity of the ELISA. If suspected, unspiked and Crustacea-spiked control matrices can be tested. Values obtained on test samples can then be adjusted accordingly. Ready-to-use standards provided with kit are meant to serve as calibrators for the assay and are NOT to be used as spiking agents. 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 and dilute the concentrated extraction buffer (**10X ExB**), sample diluent (**5X SD**), and wash buffer **(10X WB)** using distilled water or equivalent.
    - To prepare 100 ml of 1X ExB, add 10 ml of the **10X ExB** to 90 ml of distilled water. Prior to performing sample extraction, preheat the 1X ExB 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.
2. Unused 1X solutions can be stored refrigerated (2-8 °C) for up to 1 month and should be equilibrated to room temperature prior to use.
3. If buffers appear cloudy after equilibration to room temperature, they may be contaminated and should be discarded.

Preparation of samples

To ensure adequate sampling, it is important that the samples 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 finely ground food matrix or 1 mL of liquid sample to a ≥ 15 mL tube.
2. Add 9 mL of preheated 1X extraction buffer (diluted **ExB)** to mixture and briefly vortex to suspend the contents. If other starting quantities are used, a 10:1 buffer/sample ratio should be maintained.
3. Incubate tubes at 42°C (108°F) for 10 minutes, mixing vigorously every ~2 minutes.
4. Spin extraction samples at 2,000 x g for 10 minutes at room temperature and transfer the aqueous phase into a fresh tube for testing.

Swab sample extraction

1. Collection of environmental swab samples from surfaces should be carried out according to the manufacturer's protocol. For assistance with swab sample collection, please contact a BioFront Technologies representative.

2. Add 4 ml of preheated 1X extraction buffer (diluted ExB) to the swab sample collection tube containing the 1 ml swab sample solution and detached swab head. Briefly vortex to mix the contents.

3. Incubate tubes at 42°C (108°F) for 10 minutes, mixing vigorously every ~2 minutes.

4. Transfer the aqueous phase into a fresh tube for testing.

Recommended ELISA procedure

1. Determine the number of assay wells needed for test samples and for 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 to Mylar bag. Seal and store at 2-8°C.

2. Dilute sample extracts 1:2 in 1X sample diluent (1 volume of extracted sample to 1 volume diluted **SD**).

3. Add 200 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 wash, and blot dry.

6. Add 100 µL of 1X anti Crustacea-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 gently by 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, the resulting dilution factor would be two (2). Background may be subtracted to normalize data if desired.

ANALYSIS OF 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 at which the unknown sample can be compared.

Samples with normalized absorbance values above the threshold are determined to be positive, whereas those samples below the threshold are determined to be negative.

For swab samples, quantification of the target is generally not performed as the assay, is instead, intended to provide a qualitative indication as to whether or not Crustacea is present or absent in the test sample. The BioFront Technologies Surface Swabbing Kit has been validated to recover at least 1 microgram Crustacea from a 25 cm^2 area using the swab method.

Quantitative analysis of assay results

A **standard curve** should be generated from the averaged ODs of the 0-40 ppm standards after subtracting the 0 ppm averaged background values. A third order polynomial (cubic) curve fit is recommended for this evaluation. The ppm concentration of test samples can be determined by plotting OD values onto the curve and multiplying the calculated concentration by the appropriate dilution factor (if used). Note that the ppm designations on the provided standards are intended to allow the direct calculated ppm of total Crustacea in an original food sample. BioFront Technologies also offers users a MonoTrace ELISA calculation template in excel format which can be used to simplify sample quantitation. Please contact a 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 quantification is required and the OD of the test sample is above that of the 40 ppm Crustacea standard, further dilution of the sample should be performed prior to repeating the assay to ensure results fall within the assays ROQ.

ASSAY CLAIMS

When performed as instructed, the assay is capable of a simple yes/no qualitative assessment of Crustacea presence in food samples or a quantitative determination of Crustacea content. Extracted food samples that generate a colorimetric readout can be compared to the linear portion of a standard curve, allowing the interpolation of Crustacea content in ppm. The assay is capable of quantifying Crustacea content between 1 and 40 ppm.

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

SHELF LIFE

Each plate is packed in a vacuum-sealed Mylar™ pouch with desiccant and oxygen absorbing packets 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.

CUSTOMER SERVICE

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