

CRUSTACEAN KIT PRESENTATION

The ingestion of certain proteins may result in serious allergic reactions in hypersensitive individuals whether children or adults. Reactions vary from simple urticaria to fatal anaphylaxis and only avoidance may be an effective means of protecting consumers.

Among food allergens crustaceans such as shrimp, lobster, crawfish and crab are a frequent cause of adverse food reactions in allergic individuals. Traces of seafood proteins in processed foods may occur due to process cross-contamination.

The major allergen has been identified as the muscle protein Tropomyosin, a highly conserved protein found in invertebrates such as crustaceans.

Crustacean DiagnoKit™:

Quality control methodologies are required to identify whether seafood traces have made their way to the manufactured food products with no proper labeling. They are also useful to consumer protection agencies in order to enforce existing regulations on labeling of food ingredients.

Crustacean DiagnoKit™ is a competitive immunoassay allowing the detection and quantification of crustacean proteins: shrimp, crab, lobster and scampi. A specific antibody was raised targeting allergic Tropomyosins with no cross-reaction to neither chicken, pork Tropomyosins nor to other fish proteins. A suggested extraction and sample handling procedure was adapted to major food products of interest with the minimum matrix interference.

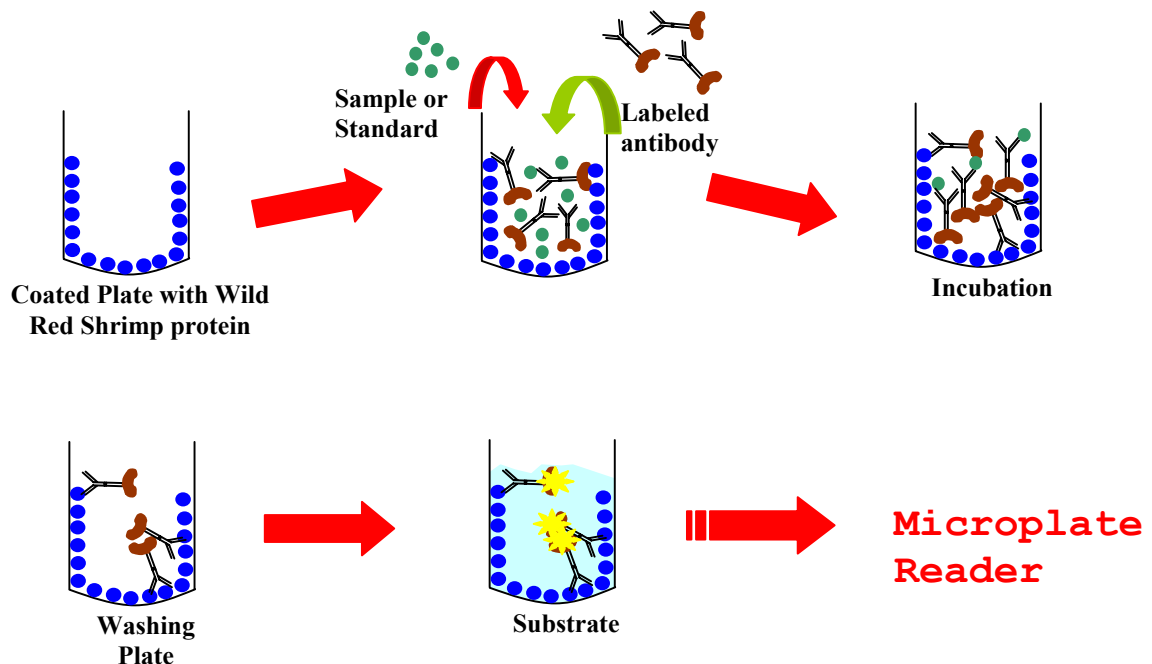


Kit Description:

- Direct enzyme-immunoassay for the quantitative analysis of Shrimp proteins invertebrate tropomyosin.
- Suggested use: prepared frozen meals, soups, fish sticks....

Test Principle:

The test is based on a competitive binding of a crustacean labelled antibody, to free and plate-immobilized proteins in a standard or sample solution.



Kit Content provided:

- Calculation diskette (MS Excel)
- 500 μL Shrimp Protein Standard ($100 \mu\text{g/mL}$)^{1,2}
- 1 microtiter plate (96 wells) coated with Crustacean proteins²
- 30 μL of shrimp antibody-HRP²
- 500 μL control sample 1²
- 1 mL control sample 2²
- 500 μL control sample 3²
- Washing buffer (dry powder)
- Dilution buffer A (dry powder, *to be used for the calibration curve and sample dilution if required*)
- Dilution buffer B (dry powder)
- 2,5 mL substrate Solution A, containing TMB
- 22,5 mL substrate Solution B, containing peroxide
- 10 mL stopping reagent, containing H_2SO_4 ³

¹Concentrations are based on a total protein amount determined by a BCA protein test

²Contains 0.05% Thimerosal as preservative, consult MSDS.

³Corrosive, use with care.



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Materials required, not provided:

- ❖ Precision adjustable pipet and a 12 or 8 channel multipipet able to deliver 200µL
- ❖ Plate reader with 450 nm interference filter
- ❖ Test tubes
- ❖ Orbital shaker
- ❖ Vortex system
- ❖ 500 mL squeeze bottle
- ❖ De-ionized water
- ❖ Timer

Extra items available upon request:

- ❖ washing buffer (dry powder) (cat.#D0310DP)
- ❖ crustacean dilution buffer A (dry powder) (cat. #D0315)
- ❖ crustacean dilution buffer B (dry powder) (cat. #D0320DP)
- ❖ extraction buffer 100mL (cat# D0330)
- ❖ protein standard solution (cat.#D0145)

Safety Precautions:

Items included in this kit are to be used by suitable qualified laboratory personnel, under proper laboratory working conditions. Handle all reagents and antibody in accordance with local safety procedures. Avoid any skin contact with stop solution and substrate B, in case of contact wash very well with water. Antibody-HRP solution contains thimerosal as preservative. Avoid contact of the reagent with the skin. MSDS (Material Safety Data Sheets) available upon request.

Procedural notes:

Store the kit at 2-8 °C. Before start the assay all reagents should be equilibrated at room temperature. Return all reagents to 2-8°C immediately after use. Do not interchange reagents between kits of different lot numbers. Do not use reagents beyond the expiration date of the kit. Substrate solution is light sensitive. Avoid exposure to direct light, and avoid contact with metal, which can cause colour development. A dark blue colour developed by the substrate solution after preparation is indicative of contamination. Sample extracts can be stored at 2-8°C for seven days and at -20°C for several months.



Preparation of Reagents

Prepare fresh diluted reagents, just prior to use

Washing Buffer:

Washing buffer is lyophilized and equivalent to 300 mL. Dissolve the dry powder in 300 mL of distilled water and store at 4°C. Vortex for obtaining a clear solution if necessary.

Dilution Buffer B:

Dilution buffer B is lyophilized and equivalent to 100 mL. Dissolve the dry powder in 100 mL of distilled water and store at 4°C. Vortex for obtaining a clear solution if necessary.

Dilution Buffer A:

Dilution buffer A is lyophilized and equivalent to 25 mL. Dissolve the dry powder in 25 mL of distilled water and store at 4°C. Vortex for obtaining a clear solution if necessary.

Standard solutions:

Standard solutions should be prepared immediately prior to use in suitable glassware. All standards are to be prepared in **dilution buffer A**.

Standard solutions for the kit may be obtained through the following dilution scheme:

Standard 1 of 20 µg/mL → 200 µL of Crustacean Standard of 100 µg/mL + 800 µL of **dilution buffer A** to obtain 1000µL

Serial dilute 1 in 4 with **dilution buffer A**:

Standard 2 of 5 µg/mL → 200µL of standard solution 1 + 600µL of dilution buffer A

Standard 3 of 1.25 µg/mL → 200µL of standard solution 2 + 600µL of dilution buffer A

Standard 4 of 0.3125 µg/mL → 200µL of standard solution 3 + 600µL of dilution buffer A

Standard 5 of 0.07813 µg/mL → 200µL of standard solution 4 + 600µL of dilution buffer A

Standard 6 of 0.01953 µg/mL → 200µL of standard solution 5 + 600µL of dilution buffer A

Standard 7 of 0.00488 µg/mL → 200µL of standard solution 6 + 600µL of dilution buffer A

Crustacean Antibody-HRP conjugate: 1/6500

1/100 → 15 µL of IgG-HRP + 1485 µL of dilution buffer B

1/6500 → 300 µL of IgG-HRP 1/100 + 19200 µL of dilution buffer B, keep this solution in freeze at 4° C.

Substrate solution A&B:

This solution should be prepared immediately prior to its use, by mixing the Substrate Solution A & B in the following proportion: 2.5 mL of Substrate Solution A + 22.5 mL of Substrate Solution B (dilution 1:10). Prepare only the needed amount of this solution. (e.g.: for 3 strips you should prepare a total of 5 mL)

Stopping solution:

Ready to use.

Control Solution 1:

Ready to use

Control Solution 2:

Ready to use

Control Solution 3:

Ready to use



Test Procedure

1. Prepare standards as described in **Preparation of Reagents**
2. Remove thimerosal from plate with washing buffer. Rinsing protocol: Fill each well to the top with washing buffer, using a squeeze bottle or multichannel pipet; turn the plate upside down and empty wells. The rinsing cycle should be carried out 3 times. Remove residual liquid by tapping the plate upside down on an absorbent paper.
3. Using a precision pipet transfer 100 µL of each standard solution for calibration into a well on the plate, and using a precision pipet transfer 100 µL of control sample 1 + 100 µL of control sample 2 and 100 µL of control sample 2 + 100 µL of control sample 3 according with the following scheme (use 100 µL of Dilution Buffer A in the Blank wells):

	1	2	3	4	5	6	7	8	9	10	11	12
A	<i>Blank 0µg/mL</i>	<i>Blank 0µg/mL</i>	Control Sample 1 + Control Sample 2	Control Sample 1 + Control Sample 2								
B	<i>Standard 0.00488µg/mL</i>	<i>Standard 0.00488µg/mL</i>	Control Sample 2 + Control Sample 3	Control Sample 2 + Control Sample 3								
C	<i>Standard 0.01953µg/mL</i>	<i>Standard 0.01953µg/mL</i>										
D	<i>Standard 0.07813µg/mL</i>	<i>Standard 0.07813µg/mL</i>										
E	<i>Standard 0.3125µg/mL</i>	<i>Standard 0.3125µg/mL</i>										
F	<i>Standard 1.25µg/mL</i>	<i>Standard 1.25µg/mL</i>										
G	<i>Standard 5µg/mL</i>	<i>Standard 5µg/mL</i>										
H	<i>Standard 20µg/mL</i>	<i>Standard 20µg/mL</i>										

Sample Wells

4. Using a precision pipet transfer 100µL of each diluted unknown sample extract into assigned well (in duplicate or triplicate).

Addition of the Antibody enzyme conjugate

5. Using a precision pipet transfer 100µL of the crustacean antibody-HRP conjugated solution, into each well, except wells: 3A, 4A, 3B and 4B.

Incubation on plate

6. Incubate the plate for 60 min. at room temperature in orbital shaker.
7. Empty the plate by inverting it over the sink then wash each well 5 times (Fill each well to the top with washing buffer, either with a squeeze bottle or a multichannel pipet. Turn the plate upside down and empty wells. The rinsing cycle should be carried out 5 times between incubation steps. Remove residual liquid by tapping the plate upside down on an absorbent paper).
8. Add 200µL of the substrate solution A+B to each well. Mix thoroughly and incubate for 20 minutes in the dark at room temperature.
9. Add 50µL of the stop solution to each well. Mix and incubate for 10 minutes in the dark at room temperature.
10. Take measurement of the absorbance with a plate reader at 450nm.



Results

You must obtain OD between 0,1 – 0,2 for control sample 1+ control sample 2, OD between 0,9-1,2 for control sample 2 + control sample 3. If your results are different please contact with us: info@abkemiberia.com

An example of data processing is presented under a Micro Soft excel format and provided in the attached disk. A calculation table allows you to tabulate the mean O.D. for a duplicate or triplicate run of standard solution. Resulting graph will be suggested.

Data is treated so as the mean value of the absorbance (450nm) readings obtained for the standards and the samples are reported to the absorbance value of the zero standard.

$$\left[\frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \right] \times 100 = \% \text{ B/Bo}$$

Maximum OD Blank = zero standard

A calibration curve can be obtained using the calculated % B/Bo value for each standard vs the log of the corresponding Protein concentration (in µg/mL).

Take the B/Bo (%) value for each sample and interpolate the corresponding concentration from the calibration curve. The linear transformation of this calibration curve may be obtained by plotting, logit (%B/Bo) vs ln C where:

$$\text{logit \% B/Bo} = \ln \left[\frac{\% \text{B/Bo}}{100 - \% \text{B/Bo}} \right]$$

➤ see provided disk, MS-Excel file

In order to obtain the unknown concentration in µg/mL contained in a sample, use one of the linear ranges of the calibration curve of your choice. The determined value must be further multiplied by the corresponding dilution factor. This is based on the assumption that the recovery after extraction is 100%.

Positives may be considered certain when the O.D. obtained for a sample is 15% lower than that of the blank solution of the calibration curves. As matrix effect may appear, dilutions of sample may be beneficial. The standard dilution buffer (Buffer A) was specially designed to mimic most commonly encountered matrices. If you dilute standard solutions on dilution buffer B the ODmax will be higher than if you use Buffer A, because no matrix effect is present on ELISA assay. Using Buffer A the ODmax must be between 0.7-1,1 .



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Suggested extraction for Crustacean

Buffers :

PBS : Phosphate Buffer Saline and Extraction Buffer

Material and Method :

1. Weigh out 10.0 g of sample into 250 mL screw top centrifuge tube.
2. Break up sample into smaller pieces
3. Add 100mL extraction buffer to each sample (100 mL for 10.0 g sample).
4. Mix together by using an homogenizer probe (Polytron) at high speed, adjust speed to prevent foaming
5. Shake samples vigorously for one hour in a heated water bath set at 45 °C.
6. Centrifuge each sample at 3,000 rpm for 5 minutes.
7. Remove supernatant
8. Centrifuge supernatant for 30 minutes at 20,000 g in a refrigerated centrifuge at 4°C.
9. Filter the extract through Whatman # 1 filter paper and refrigerate.

Extracts will remain stable over a period of seven days or can be stored at -20°C for longer periods.

NOTE: To overcome matrix effect, a 1 in 2 dilution of sample extracts is recommended. Dilution is to be made in dilution buffer B prior to performing the test.

MSDS (Material Safety Data Sheet)

Available upon request.

TECHNICAL SUPPORT:

Please write to:

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techsupport@abkemiberia.com