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Crustacean ELISA Kit Ⅱ

The Quantitative Determination for
Crustacean Protein in Food

96 Assays

For Research or Laboratory Use Only.
Not for Use in Diagnostic Procedures.
Please read full descriptions in this manual before use.

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For optimal results, follow instructions exactly. Failure to do so may lead to inaccurate results.

Reproducible results depend on careful pipetting technique, maintaining incubations at the specified temperature for the specified time, complete washing, and thorough mixing of all solutions.

INTENDED USE AND USER

The Crustacean ELISA Kit Ⅱ is a sandwich enzyme immunoassay for the quantitative determination of crustacean protein (tropomyosin as a marker for crustacean protein) in processed or unprocessed food. This kit is designed to be used by quality control personnel or other trained professionals.

Table 1 : Detectable protein in this kit

Kit	Antigen	Detectable Protein
Crustacean ELISA Kit Ⅱ	Tropomyosin	Whole Crustacean protein

PRINCIPLE OF ELISA

Crustacean protein in the samples is extracted, centrifuged and filtered prior to the sandwich ELISA. Tropomyosin (antigen) is bound to the anti-tropomyosin monoclonal antibody coated wells of the microplate module. This results in the formation of an antigen-antibody complex in the wells. Unbound materials are removed by washing. Subsequently, the enzyme-conjugated anti-tropomyosin antibody is bound to the already bound tropomyosin-antibody complex, forming an antibody-antigen-antibody sandwich. A second washing step removes the excess conjugated antibody. Addition of enzyme substrate results in color development due to the enzyme bound to the complex. After addition of the stop solution, the color intensity of the solutions can be determined by the absorbance at 450 nm. The intensity of the color developed is directly proportional to the concentration of the crustacean protein in the food. The concentration of crustacean protein corresponding to the measured absorbance is determined by preparing a standard curve, and adjusting for a further dilution factor if necessary.

KIT COMPONENTS

Label	Name of component	Content	Quantity
A	Reagent A (10X Concentrate)	100 mL	1 bottle
B	Reagent B (10X Concentrate)	100 mL	1 bottle
C	Reagent C (10X Concentrate)	100 mL	1 bottle
D	Antibody-coated Microplate Module	6X8 well modules	2 packs
E	Crustacean Standard (50 ng/mL)	1.8 mL	1 vial
F	Diluent I	100 mL	1 bottle
G	Enzyme-conjugated Antibody	13 mL	1 bottle
H	Enzyme Substrate (TMB Solution)	13 mL	1 bottle
I	Stop Solution (1N Sulfuric Acid)	13 mL	1 bottle
J	Wash Buffer (20X Concentrate)	50 mL	1 bottle
	Frame for mounting the microplate module		1 piece
	Microplate cover		1 piece

REQUIRED MATERIALS (NOT INCLUDED)

1. Distilled water (or deionized water)
2. Micropipettes and disposable tips ranging from 50 to 1000 µL
3. Graduated cylinders
4. Polypropylene centrifuge tubes (50 mL size) for sample extraction
5. Polypropylene micro tubes (1–2 mL size) for preparation of working standard solutions and dilution of sample
6. pH test paper
7. Homogenizer/blender for sample preparation (if necessary)
8. Water bath for boiling or horizontal shaker, for sample extraction
9. Centrifuge
10. Vortex mixer
11. Aspirator for washing procedure, or optional microplate washer
12. Microplate reader with a 450 nm filter, and a filter for any wavelength from 600 to 650 nm, inclusive

PERFORMANCE CHARACTERISTICS

Crustacean ELISA Kit Ⅱ is a tool to determine the presence of crustacean protein in foods.

Sample preparation time	Approx. 10 min
Sample extraction time	Approx. 30 min, or overnight
Time for ELISA analysis	Approx. 2 h
Assay sensitivity	0.31 ppm (0.31 µg crustacean protein/g food)
Detectable concentration range	0.78 to 50 ppb crustacean protein
Intra-assay precision	< 10 %
Inter-assay precision	< 10 %

REAGENT PREPARATION

Sample Extraction Solution
Prepare the ***Sample Extraction Solution*** by mixing ***Reagent A (A)***, ***Reagent B (B)***, ***Reagent C (C)*** and distilled water at a ratio of 1:1:1:7. The following example would prepare enough ***Sample Extraction Solution*** to assay 16 samples:

Reagent A (10X Concentrate) (A)	40 mL
Reagent B (10X Concentrate) (B)	40 mL
Reagent C (10X Concentrate) (C)	40 mL
Distilled water	280 mL
Total	400 mL

Note*:*
a. ***Reagent A (A)*** *may produce crystals after refrigerated storage. These crystals must be re-dissolved completely in a water bath at 30-37°C (86-99°F) prior to use. The fully re-dissolved* ***Reagent A (A)*** *can be stored at 20-30°C (68-86°F).*
b. ***Sample Extraction Solution*** *can be stored at 4°C for preparing* ***Diluent II.*** *If* ***Sample Extraction Solution*** *forms a precipitate after refrigerated storage, then the solution must be warmed in a water bath at 20-30°C (68-86°F) to re-dissolve the precipitate prior to use.*

Diluent I (F)
Diluent I (F) is used for preparing ***Diluent II*** and for diluting the ***Sample Extract***.

Diluent II
Diluent II is used to prepare ***Working Crustacean Standard***, and to further dilute the ***Working Sample Solution***.
Dilute ***Sample Extraction Solution***, 20-fold with ***Diluent I (F)***.
The following is an example of preparing ***Diluent II***:

Sample Extraction Solution	1 mL
Diluent I (F)	19 mL
Total	20 mL

Washing Solution
Dilute ***Wash Buffer (J)*** 20-fold with distilled water, and gently mix until the solution is homogeneous.

Crustacean Standard (50 ng/mL) (E)
Crustacean Standard (50 ng/mL) (E) is used as a 50 ppb standard as well as for preparing the diluted working standards described in ASSAY PROTOCOL b.

ASSAY PROTOCOL

It is strongly recommended to use disposable polypropylene tubes, and care should be taken to clean up all equipments and materials so as to be free from cross-contamination, since the assay is highly sensitive.

a. Sample Preparation

Extraction of crustacean protein from the test food sample can be performed by two different methods depending on customer's convenience.

(Short Time Extraction Method)

1. Grind and mix up the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. A disposable polypropylene centrifuge tube, containing 1.0 g of the homogenized sample and 19 mL of ***Sample Extraction Solution***, is capped tightly and vortexed for 30 seconds.
3. Incubate capped tube in a boiling water bath at 100°C (212°F) for 10 minutes.
4. Place the tube in running water to cool it down to ambient temperature (approximately 10 minutes).
5. Vortex the tube for 30 seconds.
6. Check the fluid pH with pH test paper, and neutralize (pH 6-8) with HCl or NaOH, if required.
7. Centrifuge the tube at 3,000×*g* for 20 minutes at 20-30°C (68-86°F), and the supernatant is retained as ***Sample Extract***. (Filter the supernatant with filter paper, if necessary.)
8. Dilute the ***Sample Extract*** by 20-fold with ***Diluent I (F)*** (see REAGENT PREPARATION), and the diluted solution is used as the ***Working Sample Solution*** for ELISA.
   Note*: If further sample dilution is required, dilute the* ***Working Sample Solution*** *with* ***Diluent II****.*

(Overnight Extraction Method)

1. Grind and mix up the test food sample to homogeneity with a contamination-free homogenizer/blender.
2. A disposable polypropylene centrifuge tube, containing 1.0 g of the homogenized sample and 19 mL of ***Sample Extraction Solution***, is capped tightly and vortexed for 30 seconds.
3. Fix the capped centrifuge tube to a shaker horizontally, and oscillate at room temperature overnight (for at least 12 hours at 90-110 rpm) with a reciprocating motion of about 3 cm.
4. Follow the procedures 6-8 for ***Short Time Extraction Method***.

b. Working Crustacean Standard Preparation

1. Dispense 0.5 mL ***Diluent II*** into six polypropylene micro tubes labeled 0.78, 1.56, 3.13, 6.25, 12.5 and 25 ppb.
2. Dispense 0.5 mL of ***Crustacean Standard (50 ng/mL) (E)*** into the 25 ppb tube, and mix thoroughly.
3. Dispense 0.5 mL of the freshly-prepared 25 ppb standard into the 12.5 ppb tube, and mix thoroughly.
4. Repeat this 2-fold dilution series to prepare 6.25, 3.13, 1.56, and 0.78 ppb standards.
5. Dispense 0.5 mL of ***Diluent II*** into the polypropylene micro tube labeled 0 ppb.

c. ELISA

(First reaction)

1. Unseal the ***Antibody-coated Microplate Module (D)*** after equilibrated to room temperature (20-30°C, 68-86°F). Fix the module to the mounting frame (supplied).
2. Into each well, dispense 100 µL of standards (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 and 50 ppb ***Working Crustacean Standard***) and ***Working Sample Solution***, in (at least) duplicate.
   Note*: Working Sample Solution presumed to be greater than 50 ppb should be diluted with* ***Diluent II*** *(ASSAY PROTOCOL a.8, Note)*.
3. Cover the microplate with a microplate cover (supplied).
4. Incubate for 1 hour at 20-30°C (68-86°F).

(Second reaction)

1. Remove the solution completely from each well by aspiration. (OR: Carefully pour the liquid out of the wells and tap the microplate frame upside down vigorously five times on paper towels to ensure complete removal of liquid from wells.)
2. Wash six times, by adding 300 µL ***Washing Solution*** per well followed by aspiration. After washing, remove any remaining solution by inverting and tapping the plate on a clean paper towel. (OR: Fill all the wells (300 µL of the ***Washing Solution*** in each), pour out the liquid again and tap. Repeat five more times.)
3. Add 100 µL ***Enzyme-conjugated Antibody (G)*** to each well.
4. Cover the microplate with a microplate cover and incubate for precisely 30 minutes at 20-30°C (68-86°F).

(Enzyme reaction)

1. Completely aspirate the well contents and wash six times by filling with 300 µL ***Washing Solution*** per well, and aspirate. After the sixth washing, remove any remaining solution by inverting and tapping the plate on a clean paper towel. (OR: Completely pour the liquid out of the wells, then fill all the wells with 300 µL of the ***Washing Solution***, pour out the liquid again and tap. Repeat five more times.)
2. Immediately, dispense 100 µL of ***Enzyme Substrate (H)*** into each well.
3. Cover the microplate with the microplate cover and incubate for precisely 20 minutes at 20-30°C (68-86°F). During the enzyme reaction, avoid exposing the microplate to light.
4. Stop the enzyme reaction by adding 100 µL of ***Stop Solution (I)*** to each well.
5. Immediately measure absorbance using a microplate reader at 450 nm, subtract a reference wavelength between 600 and 650 nm, inclusive.
   Note*: The absorbance must be measured within 30 minutes after stopping the enzyme reaction.*

Summary of Procedure

(Extraction procedure)

Short Time Extraction Method

Grind/mince the sample

↓

Weigh 1.0 g sample in a tube, add 19 mL ***Sample Extraction Solution***.

↓

Incubate capped tube in boiling water for 10 minutes.

↓

Cool down the tube and vortex for 30 sec.

↓

Adjust pH to 6.0-8.0.

↓

Centrifuge and filter the supernatant if necessary.

↓

Dilute ***Sample Extract*** 20-fold with ***Diluent I (F).***

Overnight Extraction Method

Grind/mince the sample

↓

Weigh 1.0 g sample in a tube, add 19 mL ***Sample Extraction Solution***.

↓

Fix the tube to a shaker horizontally, and oscillate at room temperature overnight.

↓

Adjust pH to 6.0-8.0.

↓

Centrifuge and filter the supernatant if necessary.

↓

Dilute ***Sample Extract*** 20-fold with ***Diluent I (F).***

(ELISA procedure)

Pipette 100 µL ***Working Crustacean Standard*** and ***Working Sample Solution.***

↓

Incubate the microplate for 1 hour at 20-30°C (68-86°F).

↓

Wash the wells 6 times with ***Washing Solution.***

↓

Dispense 100 µL ***Enzyme-conjugated Antibody (G).***

↓

Incubate the microplate for 30 min at 20-30°C (68-86°F).

↓

Wash the wells 6 times.

↓

Dispense 100 µL ***Enzyme Substrate (H)***.

↓

Incubate reaction for 20 min at 20-30°C (68-86°F) in the dark.

↓

Stop the enzyme reaction by adding 100 µL ***Stop Solution (I)***.

↓

Measure absorbance at 450 nm.

↓

Calculate the concentration of crustacean protein using the standard curve.

RESULTS AND INTERPRETATION

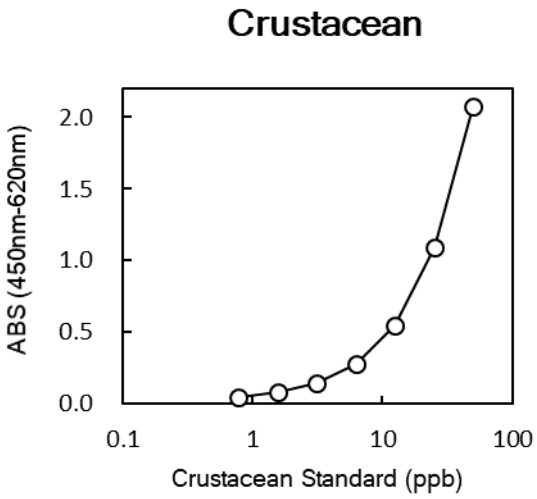
1. Determine the mean absorbance for each set of ***Working Sample Solution*** and ***Working Crustacean Standard***. Use graphing software, the built-in graphing feature of the microplate reader, or graph paper to construct a standard curve by plotting the mean absorbance for each standard on the Y axis versus the corresponding standard concentration on the X axis.
   Note*: A standard curve should be generated for each assay. For computer processing of the data, 4-parameter curve fit (Cubic regression) is recommended and a linear curve fit is also available.*
2. The crustacean protein concentration in the ***Working Sample Solution*** is interpolated from the standard curve using the mean absorbance of each observation.
3. If the mean absorbance of ***Working Sample Solution*** is greater than the absorbance of the 50 ppb standard, increase the dilution of ***Working Sample Solution*** to 40-fold or greater as appropriate instead of 20-fold, and then assay again.

The crustacean protein content in a sample, in ppm, can be estimated using the following formula:

Crustacean protein content (ppm) = OV×Dilution A×Dilution B×1/1,000

OV: Observed value (ppb)
Dilution A: Dilution for ASSAY PROTOCOL a.2, nominal 20-fold.
Dilution B: Dilution for ASSAY PROTOCOL a.8, nominal 20-fold.

TYPICAL STANDARD CURVE



STORAGE CONDITIONS AND EXPIRY OF KIT

1. Store the kit at 2-8°C (35-46°F), but DO NOT FREEZE!
2. Use the kit at 20-30°C (68-86°F), while minimizing exposure time at this temperature. The kit must be returned to storage at 2-8°C (35-46°F) as soon as possible. Do not expose this kit to temperatures in excess of 30°C (86°F)!
3. Do not use the kit after the expiration date indicated on the outside box.

WARNINGS AND PRECAUTIONS

(General)

1. Crustacean protein is used in this kit. Users who are allergic to this protein should handle kit reagents and perform the assay with great care. In case of an allergic reaction, such as sneezing or itching, seek medical attention if the reaction is serious or prolonged.
2. This product is recommended for use only by personnel trained in analytical sample handling techniques, and is meant to be used in accordance with good laboratory practices.
3. Direct contact with ***Stop Solution (I)*** should be strictly avoided. In case of contact, immediately flush affected area with copious amounts of water, and seek medical attention if necessary. In case of ingestion, call the poison control center and obtain first aid treatment.
4. Because all chemicals should be considered potentially hazardous, it is advisable to wear suitable protective clothing, goggles and gloves.
5. Because the assay is highly sensitive, all the procedures should be performed in a clean environment using uncontaminated equipment/devices and tubes/containers in order to minimize the risk of cross-contamination from previous analyses.

(ELISA)

1. Do not combine reagents from different lots or other kits.
2. All reagents should be equilibrated at 20-30°C (68-86°F) before use.
3. A standard curve should be generated for each assay concurrently with the samples.
4. Assays should be performed at least in duplicate to ensure confidence in the measured values.
5. Care should be taken to pipette standard solutions and samples accurately.
6. Follow all procedures carefully.
7. Washing must be thorough in order to minimize background readings. Complete removal of reagents from the microplate wells is essential.
8. The enzyme substrate reaction should be performed shielded from light.

Note*: In the case of processed foods, the detection efficiency or sensitivity of the assay may decrease, due to denaturation and insolubilization of crustacean proteins. Therefore, food samples that give a negative result may still contain crustacean material which is either unreactive*

or present at concentrations below the limit of detection. It should not be assumed that such foods are crustacean free.

TECHNICAL ASSISTANCE

For further technical assistance or troubleshooting advice, contact Morinaga Institute of Biological Science, Inc. or your local distributor.

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