# Crustacean Tropomyosin ELISA Kit

Enzyme Immunoassay for the quantitative determination of Crustacean Tropomyosin in food

Catalog number: ARG80793

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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## INTRODUCTION

Not only by reason of their cross-reactivity to house dust mites crustaceans represent an important group of food allergens. In this regard tropomyosin, which can be found in all common crustacean species, is the most important protein. In cooked crustacean extracts this protein represents approximately 20% of total protein.

For crustacean allergic persons hidden crustacean proteins in food are a critical problem. Already very low amounts of the allergen can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, crustacean allergic persons must strictly avoid the consumption of crustacean containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of crustacean residues in food cannot be excluded. For this reason sensitive detection systems for crustacean residues in foodstuffs are required. The Crustaceans Tropomyosin ELISA Kit represents a highly sensitive detection system for tropomyosin (from penaeus indicus) and is particularly capable of the quantification of crustacean residues in fish products, soups, dressings, bakery products and meat products.

## PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for Crustaceans Tropomyosin has to be bound onto a pre-coated microtiter plate. Tropomyosin containing samples or standards are

given into the wells of the microtiter plate. After 20 minutes incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A peroxidase conjugated second antibody directed against tropomyosin is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The absorbance is proportional to the concentration of Tropomyosin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Antibody-coated microplate | 12 strips x 8-well | 4°C |
| HRP-antibody Conjugate | 15 ml (ready to use) | 4°C |
| Standards (0; 20; 60; 200; 400 ppb of Tropomyosin) | 5 X 2 ml (ready to use) | 4°C |
| 10x Extraction & Sample Dilution Buffer | 2 X 120 ml | 4°C |
| 10x Wash Buffer | 60 ml | 4°C |
| TMB substrate | 15 ml (ready to use) | 4°C (Protect from light) |
| STOP solution | 15 ml (ready to use) | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: 620 nm as reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 10X Wash buffer or Extraction & Sample Dilution Buffer, warm to 37°C for 15 min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 20-25°C) before assay.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- The TMB Color developing agent should be colorless and transparent

before using.

- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Once the test has been started, all steps should be completed without interruption.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

## SAMPLE COLLECTION & STORAGE INFORMATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, glass vials etc. have to be cleaned thoroughly before and after each sample. Tropomyosin could adhere to different surfaces. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions.

The following sample preparation should be applied for solid samples:

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill etc.
2. 1 g of the homogenized mixture is suspended in 20 mL of **pre-diluted**

Extraction & Sample Dilution Buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at **40°C**. To ensure good homogeneity, the samples should be shaken every two minutes.

3. The samples are centrifuged for **10 minutes at 2000 g.** If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.

4. **100 µL** of particle-free solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the **pre-diluted extraction and sample dilution buffer** is necessary. The additional dilution has to be considered when calculating the concentration.

**The following sample preparation should be applied for liquid samples:**

**1 mL** of liquid sample is diluted in **19 mL** of pre-diluted **Extraction & Sample Dilution Buffer.** Afterwards the suspension is incubated for 15 min in a preheated water bath at **40°C**. To ensure good homogeneity, the samples should be shaken every two minutes. The process is continued at point 3 of solid sample extraction process.

## REAGENT PREPARATION

- **1X Wash buffer**: Dilute **10X** wash buffer into distilled water to yield 1X wash buffer. The diluted wash buffer can be stored at 4°C for at least 4 weeks.
- **1X Extraction & Sample Dilution Buffer:** Dilute **10X** Extraction buffer into distilled water to yield 1X Extraction buffer. The diluted Extraction & Sample Dilution Buffer can be stored at 4°C for at least one week.

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of **standards** and **prepared samples** in duplicate into wells.
3. Incubate for **20 minutes at RT**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X wash buffer (300 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **HRP-Antibody Conjugate** into each well. Incubate for **20 minutes at RT**.
6. Aspirate and **wash well as step 4.**
7. Add **100 µl** of **TMB substrate** to each well. Incubate for **20 minutes at room temperature** in dark.
8. Add **100 µl** of **Stop Solution** to each well.
9. Read the OD with a microplate reader at **450 nm immediately**. (optional: 620 nm as reference wave length) It is recommended read the absorbance within 30 min after adding Stop solution.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The ready-to-use standards are prepared for a direct determination of sample concentrations. The dilution of samples in the extraction process as described in the above stated sample preparation procedure is already considered. Additional dilution due to high sample concentration has to be accounted for.

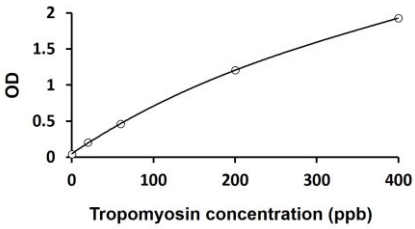
7. If these quality control criteria (The OD (450/620nm) of the highest standard (400 ppb, $OD_{400}$) should > 0.8; the standard $OD_{400}/OD_0$ should ≥ 5; $CV_0$ should <25%; $CV_{20}$, $CV_{60}$, $CV_{200}$, $CV_{400}$ should <20%) are not met the assay run is invalid and should be repeated.

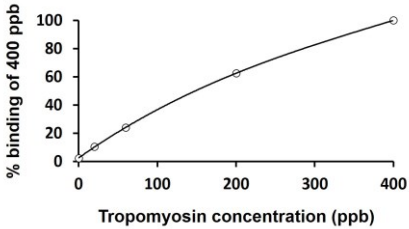
8. The determined amount of tropomyosin [ppb] can be used to calculate the amount of the corresponding crustacean raw product (dry weight). Therefore the amount of tropomyosin has to be multiplied with a conversion factor (F). The following conversion factors were determined by validation experiments:

| | |
|---|---|
| Black tiger prawns, raw | 60 |
| Black tiger prawns, cooked | 260 |
| Lobster, raw | 290 |
| Lobster, cooked | 270 |
| Crawfish, raw | 50 |
| Crawfish, cooked | 490 |
| Spiny lobster, raw | 8620 |
| Spiny lobster, cooked | 210 |
| Shrimp, raw | 70 |
| Shrimp, cooked | 70 |
| Crab, blanched | 230 |
| Crab, cooked | 520 |

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

The limit of detection (LOD) of the Crustaceans Tropomyosin test is 0.9 ppb (Tropomyosin from penaeus indicus).

Validation experiments with common matrices resulted in the following LODs [ppb]

| | |
|---|---|
| Soy sauce | 1.7 |
| Vegetable soup | 3.6 |
| Bakery products | 0.9 |
| Fish | 8.5 |
| Meat | 10.3 |

The limit of quantification (LOQ) of the Tropomyosin test is 20 ppb.

Due to the variety of sample matrices and their influence on the blank, results less than the LOQ should be treated as negative.

## Specificity

For the following foods no cross-reactivity could be detected:

| | | |
|---|---|---|
| Milk | Fish | Macadamia nut |
| Egg | Oyster | Chestnut |
| Wheat | Sunflower seeds | Pine nut |
| Rye | Pumpkin seeds | Soy |
| Oats | Cashew | Lecithin (soy) |
| Barley | Peanut | Pea |
| Rice | Hazelnut | Bean |
| Corn | Almond | Potato |
| Buckwheat | Pecan | Carrot |
| Sesame | Coconut | Leek |
| Pork meat | Brazil nut | Celery |
| Beef | Walnut | Mustard |
| Chicken meat | Pistachio | |

The following cross reactions were determined:

Cockroach (protein): 0.01%

## Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6-8% and inter-assay precision was 5-12%.

## Recovery

Mean recovery was determined by spiking samples with different amounts of tropomyosin:

| | |
|---|---|
| Soy sauce | 84% |
| Vegetable soup | 93% |
| Bakery products | 90% |
| Fish | 93% |
| Meat | 97% |