Molluscs Tropomyosin ELISA Kit

Enzyme Immunoassay for the quantitative determination of Molluscs Tropomyosin in food

Catalog number: ARG81033

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

TABLE OF CONTENTS

SECTION **Page**

INTRODUCTION 3
PRINCIPLE OF THE ASSAY 4
MATERIALS PROVIDED & STORAGE INFORMATION 5
MATERIALS REQUIRED BUT NOT PROVIDED 5
TECHNICAL HINTS AND PRECAUTIONS 6
SAMPLE COLLECTION & STORAGE INFORMATION 7
REAGENT PREPARATION 8
ASSAY PROCEDURE 9
CALCULATION OF RESULTS 10
EXAMPLE OF TYPICAL STANDARD CURVE 11
QUALITY ASSURANCE 12

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Not only by reason of their cross-reactivity to house dust mites Molluscs represent an important group of food allergens. In this regard tropomyosin, which can be found in all common Molluscs species, is the most important protein. In cooked Mollusc extracts this protein partly represents a high amount of total protein.

For Molluscs allergic person hidden Molluscs 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, Molluscs allergic persons must strictly avoid the consumption of Mollusc containing food. Cross-contamination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of Mollusc residues in food cannot be excluded. For this reason sensitive detection systems for Mollusc residues in foodstuffs are required.

The Molluscs Tropomyosin ELISA Kit represents a highly sensitive detection system for tropomyosin (from helix aspersa) and is particularly capable of the quantification of Mollusc 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 Mollusc 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 HRP 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 and the intensity of the color is measured at a wavelength of 450nm ±2nm. The absorbance is proportional to the concentration of Mollusc 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
Standard 1-5 (0; 10; 40; 100; 400 ppb of Tropomyosin)	5 X 2 ml (ready to use)	4°C
10X Extraction & Sample Dilution Buffer	2 X 120 ml	4°C
HRP-antibody Conjugate	15 ml (ready to use)	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
- Pipettes and pipette tips
- Mortar, mixer
- Water bath
- Centrifuge
- 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 Wash buffer, Extraction Buffer & Sample dilution buffer, warm to RT or 37°C for 15 min or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.
- 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 X 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 1X pre-diluted Extraction & 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 1X 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. (E.g.: 60 mL 10X wash buffer + 540 mL distilled water) The diluted wash buffer can be stored at 4°C for at least 4 weeks. If crystals are observed in the Wash buffer warm to RT or 37°C for 15 min or until the crystals are completely dissolved.
- **1X Extraction & Sample Dilution Buffer:** Dilute 10X Extraction buffer into distilled water to yield 1X Extraction buffer. (E.g.: 60 mL 10X Extraction & Sample Dilution Buffer + 540 mL distilled water) The diluted Extraction & Sample Dilution Buffer can be stored at 4°C for at least one week. If crystals are observed in the Extraction Buffer & Sample dilution buffer, warm to RT or 37°C for 15 min or until the crystals are completely dissolved.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 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 (ready-to-use) and prepared samples** in duplicate into the appropriate wells of the microtiter plate.
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, gently tap the plate to mix thoroughly.
9. Read the OD with a microplate reader at **450 nm** (reference wavelength 620 nm) immediately. It is recommended read the OD within 30 minutes.

CALCULATION OF RESULTS

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

2. Using semi-log or 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.

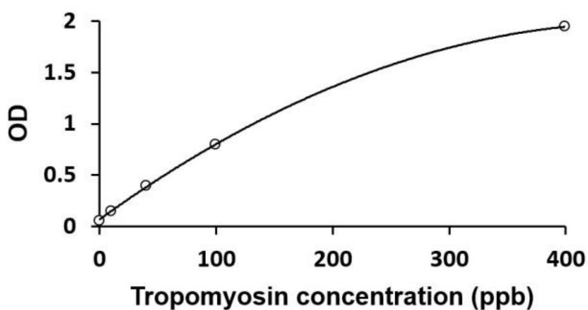
5. The determined amount of tropomyosin [ppb] can be used to calculate the amount of the corresponding Molluscs raw product (wet weight). Therefore the amount of tropomyosin has to be multiplied with a conversion factor (F). The following conversion factors were determined by validation experiments:

Abalone (Haliotis), raw	200
Abalone (Haliotis), cooked	580
Blue mussel (Mytilus edulis), raw	14990
Blue mussel (Mytilus edulis), cooked	16740
Carpet mussel (Venerupis pull.), raw	4965
Carpet mussel (Venerupis pull.), cooked	6190

Grapewine snail (Helix aspersa), blanched	550
Grapewine snail (Helix pomatia), blanched	860
Green lipped mussel (Pema canalic.), raw	8620
Green lipped mussel (Pema canalic.), cooked	11230
Oyster (Ostreidae), raw	2940
Oyster (Ostreidae), cooked	4816
Octopus (Octopoda), raw	1360
Octopus (Octopoda), cooked	1360
Scallop (Pecten jacobaeus), raw	4850
Scallop (Pecten jacobaeus), cooked	6580
Squid (Theutida), raw	700
Squid(Theutida), cooked	670

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 Molluscs Tropomyosin test is 1.7 ppb (Tropomyosin, helix aspersa).

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

Soy sauce	0.3
Vegetable soup	2.1
Bakery products	3.9
Fish	1.9
Meat	3.1

The limit of quantification (LOQ) of the Tropomyosin test is 10 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:

Almond	Cod	Pea
Apricot	Corn	Peach
Barley	Cow’s milk	Peanut
Bean, white	Cress	Pecan nut
Beef	Cumin	Pepper
Bell pepper	Egg	Pine seed
Bovine gelatin	Egg white powder	Pistachio
Brazil nut	Ewe’s milk	Plum
Buckwheat	Fish gelatin	Poppy seed
Caraway	Gliadin	Pork
Carob gum	Goat’s milk	Potato
Carrot	Guar gum	Pumpkin seed

Cashew	Hazelnut	Rice
Cayenne	Isinglass	Rye
Celery	Kiwi	Saccharose
Cherry	Lamb	Sesame
Chervil	Lentil	Soy
Chestnut	Lupin	Soy lecithin
Chicken	Macadamia	Sunflower seed
Chickpea	Mustard	Tofu
Chili	Nutmeg	Tomato
Cocoa	Oats	Walnut
Coconut	Onion	Wheat

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 5-6% and inter-assay precision was 3%.

Recovery

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

Soy sauce	74%
Vegetable soup	88%
Bakery products	100%
Fish	103%
Meat	110%

Linearity

89-107%