Fish Parvalbumin ELISA Kit

Enzyme Immunoassay for the quantitative determination of fish in food

Catalog number: ARG80797

Package: 96 wells

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INTRODUCTION

Fishes belong to the most frequent elicitors of food allergies. The allergies are predominantly induced by the low-molecular, calcium-binding muscle protein parvalbumin. The protein is characterized by its high heat resistance and stability against denaturing agents and proteolytic enzymes. Predominantly in regions with a high consumption of fish like Scandinavia, Japan or the Mediterranean countries, fish allergies represent a heavy health problem. The symptoms are ranging from inflammation of the skin over gastrointestinal and respiratory problems up to live-threatening anaphylactic shock. In spite of the high biodiversity most patients react with allergic symptoms to several fish species due to the high cross-reactivity between the fish allergens.

For fish-allergic persons hidden fish allergens in food are a critical problem. Already very low amounts of fish can cause allergic reactions, which may lead to anaphylactic shock in severe cases. Because of this, fish-allergic per-sons must strictly avoid the consumption of fish containing food. Cross-conta-mination, mostly in consequence of the production process, is often noticed. This explains why in many cases the existence of fish residues in food cannot be excluded. For this reason sensitive detection systems for fish residues in food-stuffs are required.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for fish proteins has to be bound onto a pre-coated microtiter plate. Fish 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 fish proteins 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 fish proteins. 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 0-4 (0, 4, 10, 40, 100 ppm of cod)	5 X 2 ml (Ready-to-use)	4°C
10x Extraction and 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
- Pipettes and pipette tips
- Double 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.
- Briefly spin down the HRP-Antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer and Extraction and Sample Dilution Buffer, warm to 37°C for15min, or until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards and samples be assayed in duplicates.
- In any case the ready-to-use standards provided should be determined twofold. When samples in great quantities are determined, the standards should be pipetted once before the samples and once after the samples. For final interpretation the arithmetic mean is used for calculation.
- 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 and Sample Dilution Buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°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 and Sample Dilution Buffer. Afterwards the suspension is incubated for 15 min in a preheated water bath at 60°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 Add 30 ml of 10X wash buffer in 270 ml of distilled water, mix well). Stored the diluted wash buffer at 4°C, the diluted buffer is stable for at least 4 weeks. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.
- 1X Extraction and Sample Dilution Buffer: Dilute 10X Extraction and Sample Dilution Buffer into distilled water to yield 1X Extraction and Sample Dilution Buffer. (E.g Add 30 ml of 10X Extraction and Sample Dilution Buffer in 270 ml of distilled water, mix well). Stored the diluted Extraction and Sample Dilution Buffer at 4°C, the diluted buffer is stable for at least one week. If during the cold storage crystals precipitate, the concentrate should be warmed up to 37°C for 15 minutes.

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** and **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 mixture** to each well. Incubate for **20 minutes** at **room temperature** in dark.
8. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
9. Read the OD with a microplate reader at 450 nm (reference wavelength 620 nm) immediately. It is recommended read the absorbance within 30 minutes after adding the stop solution.

CALCULATION OF RESULTS

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

2. Using log-log, 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. If the fish species of the sample is known, the amount of the appropriate species can be calculated by multiplying the test result (cod) with a species specific conversion factor:

Fish species	Conversion factor (F)
Eel	29
Flounder	7.1
Perch	5.2
Trout	2.0
Pike	0.3
Herring (smoked)	13
Carp	2.6
Salmon	1.7
Mackerel (smoked)	50
Red mullet	7.5
Shark catfish	4.2
Redfish	103

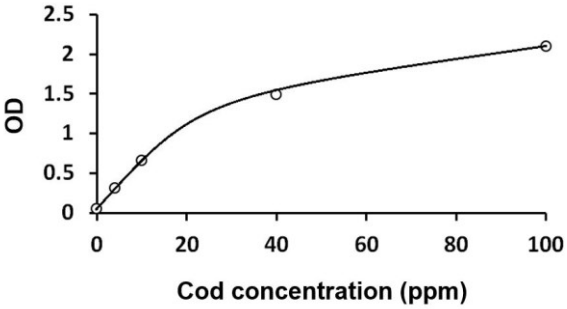
Red Snapper	29
Samlet	1.7
Sardine	101
Haddock	21
Plaice	2.4
Swordfish	1250
Hake	12
Coalfish	3.0
Devifish	274
Spined loach	32
Turbot	27
Tuna	370
Catfish	1.7
Bass	5.0
Zander	11

- It has to be considered that the standardisation as well as the conversion factors relate to fresh fish.

- For the interpretation of the test results, the grade of process of the respective food sample has to be accounted for. Validation experiments showed that cooked cod meat (20 min) resulted in a reactivity of 25% compared to fresh cod.

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 Fish test is 1.4 ppm (cod) for the standard curve. Validation experiments with common matrices resulted in the following LODs [ppm].

Wine (red)	1.5
Soup	1.3
Worcester Sauce	0.3
Asia Sauce	2.1
Cracker	0.5
Surimi	1.8
Spring Roll	1.3

The limit of quantification (LOQ) of the Fish test is 4 ppm.

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	Barley	Bean	Beef
Brazil nut	Buckwheat	Carrot	Cashew
Celery	Chicken	Corn	Egg
Fish gelatin	Hazelunt	Isinglass	Lamb
Macadamia	Milk	Millet	Mustard
Oat	Onion	Pea	Peanut
Pecan	Pistachio	Pork meat	Potato
Pumpkin seed	Rice	Rye	Scallop
Sesame	Shrimp	Soy	Sunflower seed
Walnut	Wheat		

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 7-12% and inter-assay precision was 4-10%.

Recovery

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

Wine (red)	103%
Soup	117%
Worcester Sauce	112%
Asia Sauce	103%
Cracker	99%
Surimi	114%
Spring Roll	93%

Linearity

The serial dilution of spiked samples (Wine, soup, Worcester sauce, asia sauce, cracker, surimi and spring roll) resulted in a dilution linearity of 89–105%.