CONTENT

Instruction for use

A solid-phase enzyme immunoassay for the quantitative determination of total fish I in food

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of total fish I in food.

This kit is designed for measurement of total fish I in food. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 43 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Fish proteins are among the eight major food allergens. Due to increased use of seafood in food industry, the consumers with fish allergies are at growing risk of serious reactions or even death due to mislabeled or undeclared fish derived products. Since 2004, the Food Allergen Labeling and Consumer Protection Act (FALCP) requires mandatory labeling of potential presence of this allergen in foods. The major allergens of fish muscle tissue have been located in most important fish species using specific IgE immunoblotting studies. However, due to high variability of specific IgE patterns in each species, large amount of different fish species and processing methods, it is difficult to detect all allergenic proteins of all relevant fish species by single immunoassay method. As an alternative approach, the common (marker) fish antigen can be located by immunoassays; if these antigens resist most aggressive food processing methods (high temperature cooking, repeated freeze-thaw cycles), the determination of marker antigens can indicate the potential presence of clinically relevant fish allergens in the food specimen.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific antibodies detecting common bone fish antigen. Antigens from the specimen binds to the antibodies fixed on the microwell surface. Unbound material is removed by washing procedure. Second antibodies directed towards another epitope of bone fish antigen labeled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. Avoid contact with stop solution containing 5,0% H_2SO_4. It may cause skin irritation and burns.

4.4. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.5. Do not use the kit beyond the expiration date.

4.6. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.7. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.8. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.9. Do not mix reagents from different lots.

4.10. Replace caps on reagents immediately. Do not swap caps.

4.11. Do not pipette reagents by mouth.

4.12. Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.13. The Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened / diluted components
1	SORB MTP	Total Fish I EIA strips, 8x12 wells	polystyrene microwells coated with specific anti- common bone fish antibodies	1	pcs		until exp. date
2	CAL 1–5	Calibrator set, 0.8 ml each. The set contains 5 calibrators: 0; 8; 40; 200; 1000 U/ml	common bone fish diluted in TRIS buffered preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains red dye	5	pcs	red (C1 - colourless)	2 months
3	CONJ HRP	Conjugate, 11 ml	aqueous solution of rabbit antibodies to common bone fish antigen coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and red dye	1	pcs	red	until exp. date
4	DIL	EIA buffer, 11 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative; contains blue dye	1	pcs	blue	until exp. date
5	SUBS TMB	Substrate solution, 11 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp. date
6	BUF WASH 21X	Washing solution concentrate 21x, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate - until exp.date Diluted washing solution - 1 month at +2-8 °C or 5 days at RT
7	STOP	Stop solution, 11 ml	5,0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp. date
8	N003	Plate sealing tape		2	pcs		N/A
9	K363I	Instruction Total Fish I EIA		1	pcs		N/A
10	K363Q	QC data sheet Total Fish I EIA		1	pcs		N/A

5.2. Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0.
- Preservative for samples (XEMA Cat. # S075Z) – optional
- Balance with precision of 0.1 g (for weighting products)

5.3. Storage and stability of the Kit

Store the whole kit at 2 to 8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE.

For all sample preparations, extracts (see Table M) should be cleared from particles (by sedimentation for NLT 2 hrs, or by short (3–5 min) centrifugation at 300–500 g, or by filtering through a gauze tampon or paper filter. The cleared sample should be assayed immediately or stored at +2…+8 °C for not more than 24 hrs. If XEMA Sample Buffer (Cat. #S075Z) is used, the cleared sample may be stored at +2…+8 °C up to 7 days. For longer storage, samples should be stored frozen at -20 °C or lower; in this case, samples may be stored up to 1 year. ATTENTION: avoid repeated thawing-freezing, as this may lead to unpredictable decrease in concentration of the detected antigen. After thawing, the sample should be cleared again (see above) as freeze-thaw cycle may lead to formation of aggregated particles.

7. TEST PROCEDURE

7.1. Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18 to +25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 10 wells for the calibrators CAL 1–5 and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Prepare the specimen as indicated in table M
3	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect results.
4	Pipet 50 µl of EIA buffer into each well.
5	Pipet 50 µl of calibrators CAL 1–5 and unknown samples into the wells. ATTENTION. After sample addition, the well contents may show different colors (various tints of red or violet). This is done to monitor sample addition; different colors do not influence on test performance.
6	Incubate 60 minutes at 37 °C.
7	Prepare washing solution by 21x dilution of washing solution concentrate BUF WASH 21X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times
8	Wash strips 5 times.
9	Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.
10	Incubate 60 minutes at 37 °C.
11	Wash the strips 5 times.
12	Dispense 100 µl of SUBS TMB into the wells
13	Incubate 10–20 minutes at +18...+25 °C
14	Dispense 100 µl of STOP into the wells.
15	Measure OD (optical density) at 450 nm.
16	Set photometer blank on first calibrator
17	Apply point-by-point method for data reduction.

7.5. Sample processing

An isotonic buffer solution with neutral pH (e.g., 0.1 M phosphate buffer with 0.15 M NaCl) should be used for sample preparation (extraction buffer). If extracts should be stored for more than 24 hours, it is recommended to add a preservative (e.g., sodium azide in 0.1% final concentration). We recommend to use our special Sample Preservative (Cat. # S075Z) which may be ordered separately.

For preparation of some sample types, the following disposables are required:

- cotton swabs (e.g., ear swabs)
- plastic spatula (e.g., those used for mixing of beverages)
- disposable blade or scalpel
- plastic tubes with screw caps for 15–50 ml (e.g., Sarstedt, Cat.# 60.732.001)

ATTENTION: for all sample manipulations, only disposable materials should be used. For bulky objects, sampling should be made in disposable gloves which should be changed for EACH OBJECT.

Table M

Sample type	Sample preparation method	Measuring units	Recalculation
Surfaces (cutting board, knife)	Using one swab, take the smears from 3-4 areas of the object. If local contamination is suspected, take the smears from 10–12 areas with different swabs, each swab to be used to prepare a separate sample. Submerge the swab(s) in extraction buffer (1 ml in a tube), shake the tube gently and wring the swab(s) out by pressing to the inner wall of the tube.	U/ml (of an arbitrary wash-off)	No
Frozen product	Thaw the food sample. Using a pipette, take 100 ml of the juice generated during thawing and transfer it to the vial containing 1 ml of extraction buffer.	U/ml (of an arbitrary wash-off)	No
Soft food products	Using a disposable plastic spatula, take a mixed sample from different locations, put 1.5–2.5 g of it into a pre-weighted sampling tube, weigh the sample and determine net weight of the sample. Add 8 ml of extraction buffer, close the tube with a screw cap and mix thoroughly (either by inverting or by vortexing). When testing bigger industrial samples, it is recommended to take a mixed sample of 15–30 g from different locations, put it into a disposable flask and add 80 ml of extraction buffer. In this case, mixing should be made with disposable spatula.	U/g	Obtained value/ Net weight, g
Hard food products	Using a disposable blade or scalpel, cut out thin slides of the sausage in disposable Petri dish. Put the cut sample into a pre-weighted tube, weigh the sample, determine the net weight of the sample and add 8 ml of extraction buffer. Close the tube with screw cap and mix thoroughly (either by inverting or by vortexing).	U/g	Obtained value/ Net weight, g

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

9.2. Plot a calibration curve on graph paper: OD versus pork concentration.

9.3. Determine the corresponding concentration of pork in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10. EXPECTED VALUES

	Units, U/ml	Units alternative, U/g
	Upper limit for wash-off samples	Upper limit for extracts
All types of samples	10.0	5.0

11. PERFORMANCE CHARACTERISTICS

11.1. Specificity

High specificity of the test is provided by monoclonal antibodies to common bone fish antigen (a protein included into the muscular tropomyosin complex).

The accurate quantification of the fish protein is impossible due to lacking international standard for this biochemical parameter. Therefore, currently we can provide only approximate specificity data for different fish varieties (see table below)

Specificity of XEMA Total Fish I (Bone Fish) EIA version 909.

The figures show the measured concentration of frozen-thawed muscle extract equilibrated by dry weight. The assay is calibrated by cod extract protein. The speciation of fish was made down to genus level.

Species	Systematic name (genus)	Immunoreactivity in present assay
Cod	*Gadus*	100%
Carp	*Cyprinus*	71%
Zander	*Sander*	82%
Flounder	*Pleuronectes*	66%
Sheatfish	*Pangasius*	76%
Pike	*Esox*	68%
Tilapia	*Tilapia*	81%
Hake	*Merluccius*	76%
Pollock	*Theragra*	63%
Salmon	*Salmon*	31%
Tuna	*Thunnus*	63%
Sturgeon	*Acipenser*	11%
Sharks (order)	*Selachimorpha*	<5%
Lamprey (order)	*Petromyzontidae*	<5%
Shrimp (Crustacea)	various	<5%

11.2. Repeatability. CV for the same sample within one run is not more than 8%.

11.3. Linearity. Results obtained for serial dilutions were linear (90–110% of the pre-calculated concentrations) within the range of 8–1000 U/ml.

11.4. Recovery. This parameter was estimated by testing a mixed sample (calibrators 40 and 200 U/ml, 1:1). The obtained results were within 90–110% of the due value (120 U/ml).

11.5. Analytical sensitivity. The limit of quantification, or analytical sensitivity of the test is not more than 5 U/ml for ready extract (see "Sample preparation for different sample types"). According to the results of our preliminary biochemical analysis, this value corresponds to ca. 0,5 ng/ml of the cod fish antigen. However, we recommend to express the results in arbitrary Units, as the correspondence of immunoreactivity to the weight of the target antigen is highly variable.