INSTRUCTION FOR USE

SENSI*Strip* Fish 20/5 Tests

(Cat. nr. HU0030117/HU0030157)

Lateral-flow Device for the Determination of Fish in Food and as Cleaning Control Monitoring

Sensitivity (cod) for food matrix	6 ppm
Sensitivity (cod) for swabbing	16 ng/cm^2
Sensitivity (cod) for rinse water	0.8 mg/L

1. GENERAL INFORMATION

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 persons must strictly avoid the consumption of fish containing food. Cross-contamination, 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.

The **SENSI*Strip* Fish Lateral Flow Device** represents a sensitive detection system and is based on the trans-species allergen parvalbumin. It is particularly capable to detect fish residues in food matrices, rinse water and swabs.

2. PRINCIPLE OF THE TEST

The **SENSI*Strip* Fish** test is based on the principle of immunoassay. Fish containing sample is given into a reactions vial containing an activation reagent. After 3 minutes incubation at room temperature a test strip is placed into the reaction vial. The sample migrates along the nitrocellulose membrane by capillary forces. Along its way it releases gold nanoparticles conjugated to anti-parvalbumin-antibodies. For positive samples a red line is formed when the liquid reaches the test line area. In case of negative samples, no line is formed. In any case, above the test line area a red control line appears, indicating the validity of the test. The test is evaluated after another 5 minutes.

3. PRECAUTIONS

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results:

1) Store the kit at 2-8°C.
2) Do not use the kit after its expiry date.
3) Prior to beginning the assay procedure, bring all samples and reagents to room temperature (20-25°C).
4) Extraction buffer should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
5) Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
6) Replace caps in all the reagents and samples immediately after use.
7) Use separate disposable consumables for each transfer of sample to the reaction vial in order to prevent cross-contamination.
8) Do not mix components from different batches.
9) Do not use reagents after expiration date.

NOTE: There is an expiry printed on the peel pouch of the swab. This refers to the sterility property of the devices which is not required for this application. Thus, this information must not be considered relevant.

4. KIT CONTENTS

The kit contains components and reagents for 20 tests or 5 tests. They have to be stored at 2-8°C. Expiry data are printed on the labels of the reagent containers and the outer package.

Content	20-strip	5-strip
Test Strips, in tube with desiccant stopper	20 pcs	5 pcs
Reaction vials	20 pcs	5 pcs
Extraction tubes with caps	20 pcs	5 pcs
Extraction Buffer, 60 mL, ready-to-use.	1 pcs	1 pcs
Disposable Pipettes, 0.3 mL	21 pcs	6 pcs
Disposable Pipette, 3 mL	1 pcs	1 pcs
Disposable Spatulas	20 pcs	5 pcs
Swab Sticks	20 pcs	5 pcs
Evaluation Card	1 pcs	1 pcs
Tubes and vials racks	by kit box	by kit box
QR-Code for evaluation with RapidScan ST5 lateral flow strip reader	1 pcs	1 pcs

5. SAMPLE PREPARATION

Due to high risk of cross-contamination all applied instruments like applicator, mortar, vials etc. have to be **cleaned thoroughly** before and after each sample. Allergen proteins adhere very strongly to different surfaces. In certain cases, they can resist a common dishwasher cleaning. To identify possible cross-contamination caused by previous extractions it is strongly recommended to note the sequence of the extractions for pattern recognition.

Chocolate and other products with high polyphenol content tend to show reduced results. To overcome this effect a special extraction additive can be ordered separately (HU0030100).

5.1 Solid samples / Liquid samples

1) Homogenize sample using appropriate methods depending on its specific nature (e.g. grind, crush, mix).
2) *Solid samples:* Transfer one spatula of sample to an extraction tube. Alternatively, in order to increase precision, weigh out 0.2 g of sample into an extraction tube.
   Liquid samples: Transfer a half spatula of sample liquid to the extraction tube. Alternatively, in order to increase precision, pipette 0.2 mL of sample into an extraction.
3) Add 3 mL of ready-to-use extraction buffer to the sample by using the disposable 3 mL pipette.
4) Close extraction tube with cap and shake for 1 minute.
5) Let the solid remains sediment. Depending on nature of the samples this might take 1-2 minutes. Alternatively centrifuge at 2000 g or higher.
6) Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial using a disposable 0.3 mL pipette.

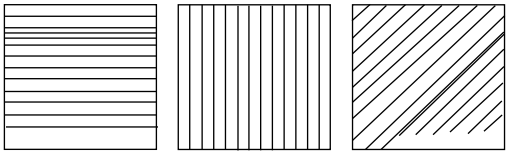
5.2 Rinse water

1) Add 2% NaCl to sample.
2) In case of strong acidic or basic rinse solution adjust the pH of the sample to 7 (+/- 0.5).
3) Transfer 0.3 mL of extraction buffer into an extraction tube using one of the disposable 0.3 mL pipettes.
4) Transfer 0.3 mL of rinse sample into the extraction tube using a second disposable 0.3 mL pipette.
5) Mix the two liquids by applying the same pipet as in step 3.
6) Transfer 0.3 mL of mixture to a reaction vial applying the same pipet as in step 4.

5.3 Swabbing samples

DRY SURFACES

1) Mark out 5x5 cm area or use swab directly on (e.g. uneven) area.
2) Transfer 1 mL of ready-to-use extraction solution into an extraction tube by using the disposable 3 mL pipette.
3) Moisten a swab by dipping into the tube.
4) Swab marked area by using crosshatch (1. horizontally, 2. vertically, 3. diagonally) technique while rotating the tip.



5) Place swab into the tube and break off the tip.
6) Close extraction tube with cap and shake for 1 minute to release the sample from the swab.
7) Remove cap and transfer 0.3 mL of sample supernatant into a reaction vial using a disposable 0.3 mL pipette.

WET SURFACES

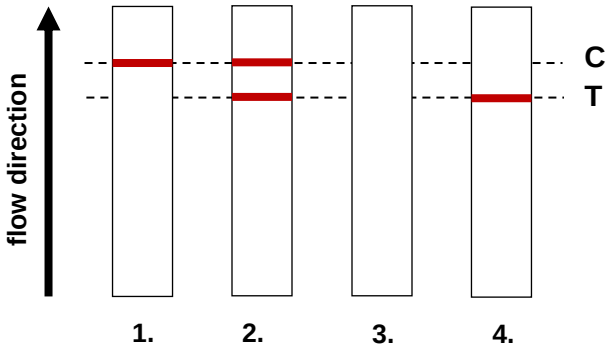
Apply same method as described for dry surfaces <u>without</u> prior need to moisten the swab.

6. ASSAY PROCEDURE

1) Prepare samples as described above.
2) After transfer of the sample to the incubation vial add cap and shake for 15 seconds. Make sure that the biotinylated antibody is completely dissolved.
3) Incubate for 3 minutes.
4) Remove cap and place one strip into the vial. For proper strip orientation make sure that the arrows on the cover foil point downwards.
5) Incubate for 5 minutes.
6) Remove strip from the vial and evaluate immediately.

7. EVALUATION

SENSI*Strip* lateral-flow devices are evaluated according to the following scheme:



1. **Negative:** visible control (C) line, no test (T) line
2. **Positive:** visible control (C) and test (T) lines
3. **Invalid:** neither control (C) and test (T) lines visible
4. **Invalid:** no control (C) line and visible test (T) line

For a better distinguishing between negative, borderline and positive samples a colour card for evaluation is provided with the kit. The intensity of the test line has to be compared with the different increments of the colour card. Results lower than increment 3 should be treated as negative. Results according increment 3 or higher should be treated as positive. Since the increments of the colour card are ranging up to 10 a semi-quantitative evaluation is also possible. This can be improved by taking into account the results stated in the validation report of the product.

In addition, a quantitative evaluation (6 - 100 ppm) in combination with the *Gold Standard Diagnostics RapidScan ST5* lateral flow reader is possible. For further information, please contact Gold Standard Diagnostics.

8. PERFORMANCE

8.1 Sensitivity

LOD (cod) of the SENSI*Strip* lateral-flow test is 6 ppm for food matrix, 0.8 mg/L for rinse water and 0.016 µg/cm^2 for swab samples applying the procedure above. The corresponding amounts of the individual fish species can be estimated by taking into account the results stated in the validation report of the product.

NOTE: Sensitivity may vary depending on matrix and processing of a complex food mixture. For achieving reliable results each matrix should be validated prior to routine testing.

8.2 Cross-reactivity

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

Adzuki bean	Cumin	Oyster
Almond	Curcuma	Paprika
Apricot	Dill	Pea
Barley	Duck	Peach
Bean, white	Egg white	Peanut
Beef	Ewe's milk	Pepper
Brazil nut	Fennel	Pine nut
Bovine gelatin	Fenugreek	Pistachio
Pecan nut	Fish gelatin	Poppy seed
Buckwheat	Flaxseed	Pork
Caraway	Garden cress	Potato
Cardamom	Garlic	Pumpkin seed
Carob bean	Gliadin	Radish
Carrot	Goat's milk	Rice
Cashew	Guar gum	Rye
Cayenne	Hazelnut	Sesame
Celery	Horseradish	Shrimp
Cherry	Kidney bean	Soy flour
Chestnut	Kiwi	Soy lecithin
Chia	Lamb	Soy milk
Chicken	Leek	Split peas
Chickpea	Lentil	Sucrose
Chili	Lupin	Sunflower seed
Cinnamon	Macadamia	Thyme
Clove	Milk powder	Tomato
Cocoa	Mustard, yellow	Turkey
Coconut	Nutmeg	Walnut
Corn	Oats	Wheat
Cow's milk	Onion	White cabbage

8.3 High-dose-hook Effect

Reduced or absent signals can occur in case of very high concentrations. The test gives valid results up to a concentration of 25000 ppm for food samples, according 66.7 µg/cm^2 for swabs and 3333 mg/L for rinse water samples.

8.4 Additional Performance Data

Additional data can be found in the corresponding validation report of the product, which can be inquired at Gold Standard Diagnostics.

9. LIABILITY

Gold Standard Diagnostics Budapest shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.

Gold Standard Diagnostics Budapest shall not be liable for the unsafe use of the kit out of the current European safety regulation.