



SYMMETRIC HISTAMINE

Lateral Flow Test Kit

for the quantitative determination of Histamine in Fresh or Thawed Frozen Raw Fish Samples and Canned fish

This Lateral Flow test kit is manufactured by ProGnosis Biotech S.A.

ProGnosis Biotech S.A. is ISO 9001:2015 certified by TÜV Hellas (TÜV NORD).

Use only the current version of Product Data Sheet enclosed with the kit.

Symmetric Histamine, S9030/S90180, is a lateral flow test kit for the quantitative determination of Histamine, in fresh fish, canned fish and frozen fish. This kit contains all reagents required for 30/180 reactions.

Matrices:

Type I: Fresh or Thawed Frozen: Tuna, tuna related species, anchovy, bonito, bluefish, cod, mackerel,

mahi mahi, marlin, sardines, salmon flour. Canned: Tuna, tuna related species, anchovy, bonito, bluefish, cod, mackerel, mahi mahi, marlin, sardines.

- Sample preparation: extraction
- Test time (incubation time after samples and reagents preparation): 3min
- Range: 8 - 300ppm
- Assay running temperature: 21– 25°C
- Shelf life: 12 months
- Storage: 15-25°C

Specifications

- The LOD of the method is 5.3ppm.
- The LOQ of the method is 8ppm.
- Cross Reactivity

X-reactive compound	Cross Reactivity (%)
Histamine	100
3-methylhistamine, Tyramine, Tryptamine, Putrescine, Histidine, Cadaverine, Spermine, Trimethylamine, Serotonin, L-tryptophan, L-phenylalanine, L-histidine, L-tyrosine	<0.01

1. Description

Symmetric Histamine is an innovative Lateral Flow test, utilizing state-of-the-art features for the quantitative detection of Histamine in Fresh or Thawed Frozen Raw Fish Samples and Canned fish.

2. General Information

Histamine (HA) is a heterocyclic primary amine and is the by-product of the decarboxylation of the amino acid L-histidine by the enzyme histidine decarboxylase. The latter is produced by certain bacteria that can be found in sea food. The most common fish species that tend to accommodate HA belong to the scombroid family of fish (tuna, mahi mahi, marlin, bluefish, sardines, anchovy, bonito, herring and mackerel). After the fish are harvested the bacterial growth results in increasing histidine decarboxylase amounts which in turn induces HA production. High levels of HA may cause scombroid poisoning in humans which consists of various symptoms, such as rash, nausea, vomiting, diarrhea, hypotension, heart palpitations and muscle weakness. Most controlling government agencies worldwide have regulations regarding the amount of HA allowable in fish and fish products. HA levels in fish of good quality are usually below 10 ppm. HA values between 50 and 200 ppm are acceptable depending on the fish species and the country. Accurate and rapid determination of HA presence in commodities is of paramount importance.

3. Principle of the method

The Symmetric Histamine kit is a novel quantitative lateral flow device based on immunochromatography assay principles where the target analyte, HA, binds to histamine specific antibodies conjugated to colloidal gold. HA is extracted from seafood samples with distilled or deionized water and a diluted extract is added into a test tube. A dipstick with two capture lines, test and control, is dipped into the test tube and the suspended mixture starts flowing vertically on the dipstick passing through the two lines. While running, HA (if it is present) binds to the antibodies. A valid test should always have the upper control line red. If the sample is free of HA, a color development occurs at the test line, indicating the absence of HA in the sample. On the contrary, the presence of HA in the sample will cause a reduced colored signal at the test line. The test line color intensity is indirectly proportionate to the concentration of HA present in the samples. By utilizing S-Flow software and the symmetric quantification technology HA is accurately quantified [1,2].

4. Reagents Provided

- Lateral flow test sticks— 30/180 tests, ready to use
- Sample Diluent tubes— 30/180 pre-diluted tubes
- Clear plastic test tubes— 30/180 tubes
- Product's instructions manual
- Product data sheet

5. Materials required but not provided

- A grinder sufficient to render sample homogenized
- Balance with 0 - 50 g measuring capability and Graduated cylinder - 100 mL
- Distilled or deionized water
- Filter Paper Whatman #1, Filter Funnel and Miscellaneous laboratory plastic tubes 50 - 125 mL
- Vortex mixer and/or blender/homogenizer
- 100 or 200 μ L adjustable single channel micropipettes with disposable tips
- pH strips or pH meter
- NaOH and/or HCl
- S-Flow software along with matching scanner device provided by lateral logic ltd

6. Storage Instructions

Store kit components between 15 - 25°C. Do not freeze any components provided. Reseal the unused strips in the storing tube together with the desiccant bag provided. The expiry date of the kit and reagents is stated on their labels and no quality guarantee is accepted after the expiration date. The expiry of the kit components can only be guaranteed if the components are stored properly and the reagent is not contaminated due to prior handling. Do not interchange individual components between kits of different lot numbers.

7. Safety and Precautions for use

Cover or cap all reagents when not in use. Use a clean disposable plastic pipette tip for each reagent, to avoid cross contamination.

8. Sample Preparation

8.1 Fresh or Thawed Frozen Raw Fish Samples

- A. Clean and eviscerate three fish.
- B. Cut three cross-sectional pieces 2.5 cm (1 in.) thick, from back of the pectoral fin, halfway to vent and one posterior to the vent.
- C. Debone slices and blend or grind combined samples until homogenous. Store samples at 2–8°C (35–46°F) until analysis, and ensure they are analyzed within no more than 3 hours.
- D. Add 10 g of the homogenous mixture to a clean disposable extraction bottle containing 190 mL distilled or deionized water.
- E. Close the bottle and vortex for a minimum of 1 min.
- F. Allow tissue to settle to the bottom of the bottle for about 30 sec, filter 5 - 10 mL of the extract through a Whatman #1 filter paper and collect the filtrate.
- G. The extracted sample should have pH value of 6.2 - 7.5. If the pH is less than 6.2 or more than 7.5, it should be neutralized using NaOH or HCl.
- H. Add 100 μ L of filtrate into the Sample diluent tube provided and mix well.
- I. In case the sample contains more than 300ppm an additional dilution with deionized water is suggested as shown in the table below.

8.2 Canned Fish

- A. Place entire contents of can, meat, and liquid, into a blender. Blend until homogenous. Store samples at 2–8°C (35–46°F) until analyzed, and ensure they are analyzed within no more than 3 hours.
- B. Add 10 g of the homogenous mixture to a clean disposable extraction bottle containing 190 mL distilled or deionized water.
- C. Follow the extraction and dilution procedure as described in step 8.1.

Filtrate volume	Distilled or Deionized Water volume	Dilution Factor	Range of quantification	LOQ
1000 μ L	4000 μ L	5	0 - 1500 ppm	40
1000 μ L	9000 μ L	10	0 - 3000 ppm	80

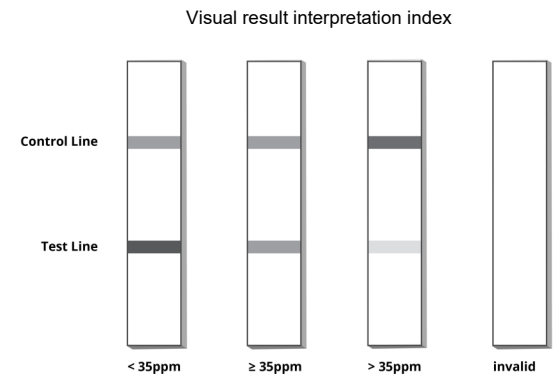
NOTE: Use only plastic vials (e.g. polypropylene, PP) for the sample preparation. HA adsorbs on glass surfaces which leads to reduced recoveries.

9. Method procedure

1. **The recommended assay running temperature is 21-25°C.**
2. Remove the required amount of LFD test sticks from storage container for the number of test portions to be analyzed.
3. The pot with dipsticks should **always be well closed** after reagents have been taken out.
4. Dispense **100 μ L of the diluted filtrate** into the clear plastic test tube.
5. Immerse the dipstick into the test tube following the direction shown by the arrows, so that the uncovered area of the dipstick gets soaked. **Note:** In case the dipstick gets inserted in the wrong direction (arrows pointing up) and gets wet at the top label area, it becomes useless and has to be replaced with a new dipstick.
6. When the 3 minutes are over, take the dipstick of the test tube.
7. Remove the white cotton sample-pad of the stick. Hold the stick from the top and remove the white sample-pad with your hands. Do not use a paper towel or any other material.
8. Place the stick inside the plastic holder in order to be scanned.
9. Use S-flow software to quantify results within 1 minute after the end of analysis. The software will use a Lot specific curve to calculate the results (ppm) according to the matrix sample type.

10. Visual Interpretation

When the test time is completed (3 min), the dipstick can also be visually read and interpreted according to the following figure.



1. If the test line is darker than the control line (T>C), the sample contains Histamine lower than 35 mg/kg.
2. If the test line is the same with the control line (T=C), the sample contains Histamine at the regulatory limit or higher (\geq 35 mg/kg).
3. If the test line is much lighter than the control line (T<C), the sample contains Histamine higher than 35 mg/kg.
4. The control line should always be visible, if not the test is invalid.

Note: 35 mg/kg is the qualitative cutoff for histamine detection using the Symmetric Histamine kit. Samples yielding visual results near this threshold (just below or above) are recommended to be re-tested or confirmed using a quantitative method.

11. Performance Evaluation

11.1 Reference Materials

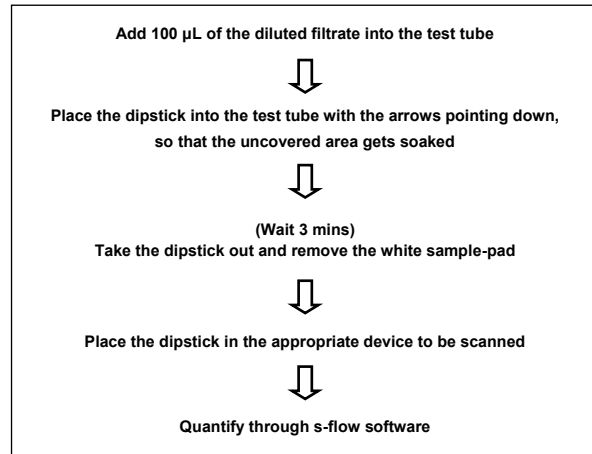
Several reference materials are being used for the evaluation of each product of ProGnosis Biotech S.A. in the context of Quality Control performed by Quality Control Department. Please request a validation report, including the results, at exports@prognosis-biotech.com.

12. References

- [1] Papageorgiou G, Ntantisios AN, Voulgari D, Badra K, Gotsopoulos M and Athanasiou SD, An innovative symmetric lateral flow system for the quantification of Aflatoxin M1. 8th International Symposium on RAFA, 7-10 November 2017, Prague, Czech Republic.
- [2] Ntantisios AN, Arampatzis A, Voulgari D, Badra K, Papageorgiou G, Athanasiou SD and Gotsopoulos M, Innovative lateral flow method for the quantification of Aflatoxin M1. IDF DAIRY SUMMIT, 29 October-03 November 2017, Belfast, Northern Ireland, UK.

13. Method Summary

Total method time: 3 minutes.



All immune assays supplied by ProGnosis Biotech S.A., are warranted to meet or exceed our published specification when used under normal conditions in your laboratory. If the product fails during the stated period, a replacement product will be issued.

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LATERAL FLOW TEST KIT

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www.prognosis-biotech.com
e: exports@prognosis-biotech.com
t: +30 2410 623922
Farsalon 153 | 41335 Larissa, Greece

