Histamine ELISA Kit

Catalog No: E-FS-E182

96T/96T*3

Version Number:	V1.1
Replace version:	V1.0
Revision Date:	2026.03.02

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

The kit is an enzyme-linked immunosorbent assay (ELISA)designed for the quantitative determination of Histamine. The specificity of the kit has been evaluated by analyzing cross-reactivity with related substances. It offers a convenient,rapid,and sensitive method for large-scale screening of histamine in fresh and thawed frozen fish and shrimp.

Technical indicator

Reaction mode (Incubation time and temperature) **:** 25℃; 15 min~30 min

Detection limit: ---2.5 ppm.

Sensitivity: ---0.03 ppm

Specificity: Histamine---100%

Sample recovery rate: --- 80% - 120%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Standards	1.5 mL each (ppm=mg/mL=mg/kg) (0 ppm, 0.03 ppm, 0.09 ppm, 0.27 ppm, 0.81 ppm, 2.43 ppm)
Histamine Standards (100 ppm)	1 mL
Enzyme Conjugate Buffer	7 mL
11× Enzyme Conjugate Concentrate	1 mL
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20× Concentrated Wash Buffer	25 mL
Sample Diluent	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other supplies required

Instruments: Microplate reader, Homogenizer, Nitrogen evaporators, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility 0.01g).

Micropipette: Single channel (20-200 μL, 100-1000 μL), Multichannel (30-300 μL).

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Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E182. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E182 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**

   Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Enzyme Conjugate Working Solution:

Dilute the **11× Enzyme Conjugate Concentrate** with **Enzyme Conjugate Buffer**. (11×Enzyme Conjugate Concentrate (V): Enzyme Conjugate Buffer (V) =1:10).

Wash Buffer

Dilute **20× Concentrated Wash Buffer** with deionized water. (20× Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

Substrate Solution:

Mix **Substrate A** Solution with equal volume of **Substrate B** Solution. Use it within 5 min. (To prevent contamination, do not return any substrate to the original container.Substrate solutions showing coloration should be discarded as they are indicative of deterioration.)

3. Sample pretreatment procedure

3.1 Fresh and thawed frozen Fish, Shrimp

(1) Weight 1±0.05 g of sample to a 15 mL centrifuge tube.
(2) Add 10 mL of **Distilled water**, then mix vigorously (vortex) for 5 min.
(3) Centrifuge at 4000 g for 5 min at room temperature..
(4) Transfer 100 µL sample supernatant into a new 2 mL centrifuge tube.
(5) Add 400 µL Distilled water or **Sample Diluent** (Distilled water for shrimp, **Sample Diluent** for fish), mix thoroughly for 30 s.
(6) Pipette the aqueous layer for asay (without furiher dilution).

Note: Sample dilution factor: 50, detection limit: 2.5 ppm

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add Sample:** add 50 μL of **each Standard or Sample** per well, then add 50μL of **Enzyme Conjugate Working Solution** to each well, cover the plate with plate sealer, oscillate for 5 sec gently to mix thoroughly, incubate at 25℃ for 20 min in shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 260 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 4 times, 30 sec intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **Color Development:** add 100 μL **Substrate Solution** into each well. Gently oscillate for 5s to mix thoroughly. Incubate at 25℃ for 10 min in shading light.
5. **Stop Reaction:** add 50 μL of **Stop Solution** to each well, oscillate gently to mix thoroughly.
6. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 5 min after stop reaction.

Result analysis

1. **Absorbance (%) = A/A_0×100%**

   A: Average absorbance of standard or sample

   A_0: Average absorbance of 0 ppb Standard

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add average absorbance value of sample to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.**

   For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

Histamine Standard Curve (E-FS-E182)