

PRECAUTIONS

1. Store test kit between 2-8°C (35-46°F) when not in use.
2. Do not use kit components beyond expiration date.
3. Glassware should not be used for extraction purposes. Because histamine may adhere to glass, using glassware may affect test results.
4. Do not mix reagents from one kit serial with reagents from a different kit serial.
5. Do not run more than 6 wells per test, unless using a multichannel pipettor.
6. Follow proper pipetting techniques, including priming tips by filling and dispensing solution once before use.
7. Use of incubation times other than those specified may give inaccurate results.
8. Kits should be brought to 68-82°F prior to use.
9. Avoid prolonged storage of kits at ambient temperatures.
10. Do not freeze test kits.
11. To avoid cross-contamination, use clean pipette tips for each sample.
12. Commodity extracts should have a pH of 6-8 before testing. Excessively acidic or alkaline samples should be adjusted. For instructions on adjusting pH, contact Neogen.

PROCEDURAL NOTES

1. **Substrate.** K-Blue Substrate is ready for use. The substrate should be clear to a very light blue—discard if it has turned dark blue. Only pour the needed volume of substrate into a reagent boat. **Do not return unused substrate to the bottle.** Cover the reagent boat to keep the substrate protected from light until it is needed.
2. **Control.** The control provided with the kit is ready to use. Do not dilute the control in the same manner as sample extracts. This control is equivalent to a concentration of 15 ppm in a properly extracted sample.
3. **Sample extract diluent buffer.** Prepare by adding foil pouch of extract buffer to 1.0 L of distilled or deionized water. Swirl to mix. Store remaining buffer covered at room temperature.
4. **Wash buffer.** The wash buffer is supplied as a 25X concentrate. Prepare by mixing wash buffer concentrate (40 mL) to 1.0 L of distilled or deionized water. Swirl to mix. Do not shake. Store remaining wash buffer at room temperature.
5. **Antibody wells.** Keep wells sealed in the foil pouch until needed. Remove wells from the foil pouch only after the samples are extracted, and the test procedure is set to begin.

SAMPLE PREPARATION

1. **Canned tuna:** AOAC 937.07b - Place entire contents of can or pouch, meat and liquid, into a blender. Blend until homogenous.
2. **Fresh or thawed frozen raw fish:** AOAC 937.07a - Clean and eviscerate three fish. Cut three cross-sectional pieces 2.5 cm (1 inch) thick, from back of the pectoral fin, halfway to vent and one posterior to the vent. Debone slices and blend or grind combined samples until homogenous.

SAMPLE EXTRACTION AND DILUTION

Store samples at 2-8°C (35-46°F) until analyzed. **Note:** The following extraction instructions may be used whether using Neogen's Alert for Histamine Extraction Kit, or your own extraction materials.

1. Add 10 grams of the homogenous mixture to a clean blender jar containing 115 mL of distilled or deionized water. Blend at high speed for 1 minute, and allow the sample to settle before proceeding to step 2.
Alternative Shaking method:
 - a. Add 10 grams of the homogenous mixture to a clean disposable extraction bottle containing 115 mL of distilled or deionized water.
 - b. Tightly cap and vigorously shake the bottle for 15 to 20 seconds to suspend the fish tissue in the water.
 - c. Wait approximately 5 minutes, then shake the bottle for 15 to 20 seconds to resuspend the fish tissue.
 - d. Wait an additional 5 minutes, and again shake the bottle for 15 to 20 seconds to resuspend the fish tissue. Allow the tissue to settle to the bottom of the bottle for about 30 seconds.
2. Allow sample to settle or filter using folded filter paper or Neogen filter syringe.
3. Add 5 mL of sample extract diluent buffer to a clean test tube or bottle.
4. Using a clean pipette tip, add 100 µL of the supernatant or filtered extract to the sample extract diluent buffer.
5. Vortex or swirl to mix. The sample is now ready to test. Repeat for all samples.

TEST PROCEDURE

Note: Allow all reagents to warm to room temperature, 68-82°F, prior to use.

1. Remove 1 red-marked mixing well for each sample to be tested plus 1 red-marked well for the control, and place in the well holder.
2. Remove an equal number of antibody-coated wells. Return antibody wells which will not be used immediately to the foil pack with desiccant and reseal to protect the antibody. Mark one end of strip with a "1", and place strip in the well holder with the marked end on the left. Do not mark on the inside or bottom of the wells.
3. Mix each reagent by swirling the reagent bottle prior to use.
4. Pipette 100 µL of conjugate from the blue-labeled bottle into each red-marked mixing well.
5. Using a new pipette tip, add 100 µL of the control from the yellow-labeled bottle to the first red-marked mixing well. Mix the control with the conjugate in the well by pipetting it up and down 5 times. Discard the used tip.
6. Using a new pipette tip, add 100 µL of diluted sample to the next red-marked mixing well. Mix as in the previous step. Record the location of each sample for identification. Repeat the process for each sample, using a new pipette tip for each.

7. Using a new pipette tip for each, transfer 100 µL from each red-marked mixing well to an antibody-coated well (a 12-channel pipettor should be used for more than 6 wells). Timing is very important with the transfers. All transfers must be completed in under a minute, or the accuracy of the subsequent results may be jeopardized.
8. Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing reagents from the wells. Incubate for **10 minutes** at 68-82°F. Discard red-marked mixing wells.
9. Shake out the contents of the antibody wells. Wash the antibody wells by completely filling the wells with diluted wash buffer and dumping them out. Repeat this wash step 3 more times. Following the last wash, turn the wells upside down and tap out the remaining liquid on an absorbent towel.
10. Add 100 µL of substrate from the green-labeled bottle to each antibody well (a 12-channel pipettor should be used for more than 6 wells).
11. Mix by sliding back and forth on a flat surface for 10-20 seconds. Incubate for **10 minutes** at 68-82°F.
12. Add 100 µL of Red Stop from the red-labeled bottle to each well in the same order as the substrate.

INTERPRETATION OF RESULTS

If a sample well is a **darker blue** than the control well, the sample contains **less than 15 ppm** of histamine. If a sample well shows **less blue** color, or **more red** color, than the control, the sample contains **more than 15 ppm** of histamine. For optimum observation of color differences, place the wells on a white surface and read looking down through the solution.

Alternative: Microwells may be read in a microwell reader. Wipe bottom of wells, blank reader on air using a 650 nm filter, and compare sample readings to 15 ppm control reading.

RETESTING AND LIMITATIONS

If positives occur in commodities not previously tested, confirm with an additional approved method prior to taking action. The results obtained when using a 10 gram sample may not be representative of the entire sample. The results are qualitative and should be used for screening only.

VALIDATED MATRIXES

Tuna, mahi-mahi, bluefish and fishmeal.

CUSTOMER SERVICE

Customer assistance and Technical Services for Alert for Histamine can be reached by calling 800/234-5333 (USA/Canada) or 517/372-9200, 8 a.m. to 7 p.m. Eastern time. Assistance is available on a 24-hour basis by calling 517/334-0460. Training on this product, and all Neogen test kits, is available.

MSDS INFORMATION AVAILABLE

Material safety data sheets (MSDS) are available for this test kit, and all of Neogen's Food Safety test kits, on Neogen's Web site at www.neogen.com, or by calling 800/234-5333 or 517/372-9200.

WARRANTY

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TESTING KITS AVAILABLE FROM NEOGEN**Natural Toxins**

- Aflatoxin, DON, Ochratoxin, Zearalenone, T-2 Toxin, Fumonisin, Histamine

Foodborne Bacteria

- *E. coli* O157:H7, *Salmonella*, *Salmonella enteritidis*, *Listeria*, *Listeria monocytogenes*, *Campylobacter*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*

Sanitation

- ATP, Yeast and Mold, Total Plate Count, Generic *E. coli* and Total Coliforms, Protein Residues

Food Allergens

- Peanuts, Milk, Eggs, Almond, Gliadin, Soy Flour, Hazelnut

Genetic Modification

- CP4 (Roundup Ready®)

Ruminant By-products

- Meat and Bone Meal, Feed



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Read instructions carefully before starting test

ALERT®

Histamine Screening Test Bulk Kit

REFRIGERATE AT 2-8°C — DO NOT FREEZE

HISTAMINE

High levels of histamine may develop in a variety of fish species as they decompose. These species include tuna, mahi-mahi, marlin, bluefish, sardines, anchovy, bonito, herring and mackerel. Ingestion of histamine may cause scombroid poisoning in humans, which may lead to a variety of symptoms, including rash, nausea, vomiting, diarrhea, hypotension, palpitations and muscle weakness. Paralysis and death have also been reported in cases of scombroid poisoning.

Because of its potential for human illness, the U.S. Food and Drug Administration (FDA) has ruled that extensive refrigeration records and/or histamine testing must be included in a Hazard Analysis Critical Control Point (HACCP) program for relevant fish species. The FDA has set an action level of 50 parts per million (ppm) for histamine in domestic and imported fish.

INTENDED USE

Alert for Histamine is intended for the qualitative analysis of histamine in scombroid species of fish, such as tuna, bluefish and mahi-mahi.

INTENDED USER

The test kit is designed for use by quality control personnel and others familiar with histamine analysis in fish. Since technique is very important, operators should be trained by a Neogen representative or someone who has completed Neogen training.

STORAGE REQUIREMENTS

The kit can be used until the expiration date on the label when stored refrigerated at 2-8°C (35-46°F).

ASSAY PRINCIPLES

Alert for Histamine is a competitive direct enzyme-linked immunosorbent assay (CD-ELISA). Histamine is extracted from a sample in a quick water extraction process. This extract is filtered and then diluted into a buffer solution supplied with the test. Free histamine, in the buffered sample and control, is allowed to compete with enzyme-labeled histamine (conjugate) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound enzyme conjugate to produce blue color. The color of the sample is visually compared to the color of the control. If the sample has more blue color than the control, it contains less histamine than the control. If the sample contains less blue color (or more red) than the control, it contains more histamine than the control.

MATERIALS PROVIDED

1. 24 12-well antibody-coated strips
2. 24 12-well red-marked mixing strips
3. 12 yellow-labeled bottles of 15 ppm histamine control
4. 2 blue-labeled bottles of histamine-HRP conjugate solution
5. 6 foil pouches of sample extract diluent buffer concentrate of 10 mM PBS dry powder
6. 2 bottles of 40 mL wash buffer concentrate of 250 mM PBS-Tween
7. 2 green-labeled bottles of K-Blue® Substrate solution
8. 2 red-labeled bottles of Red Stop solution

MATERIALS RECOMMENDED BUT NOT PROVIDED

1. Distilled or deionized water
2. Graduated cylinder, 100 mL
3. Neogen filter syringe, Whatman #1 filter paper, or equivalent
4. Sample collection tubes
5. Blender and plastic blender jars or disposable bottles \geq 150 mL
6. Scale capable of weighing 10-50 grams (Neogen item #9427)
7. Pipettor, 100 μ L or equivalent (Neogen item #9272, #9276, or #9278)
8. Pipettor, 12-channel (Neogen item #9273)
9. Pipette tips for 100 μ L and 12-channel pipettors (Neogen item #9410)
10. Reagent boats for 12-channel pipettor (Neogen item #9435)
11. Paper towels or equivalent absorbent material
12. Microwell holder (Neogen item #9402)
13. Timer (Neogen item #9426)
14. Waterproof marker
15. Wash bottle (Neogen item #9400 or #9366)