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MAX SIGNAL®

# Histamine Enzymatic Assay Kit Manual

**Catalog #: 1032**

***Reference #: 1032-04***

ISO 9001 QUALITY ASSURANCE

This kit is manufactured to the international quality standard ISO 9001:2008.
ISO CI#: SARA-2009-CA-0114-01-B

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# GENERAL INFORMATION

## Product Description

The *MaxSignal® Histamine Enzymatic Assay Kit* is a colorimetric enzymatic assay for the determination of histamine in fresh fish/seafood, fish meal, wine and milk. Histamine is a contaminant sometimes found in seafood when improperly handled. High levels of histamine in seafood can cause scombroid poisoning. Quality tuna has histamine levels below 50 ppm. The amount of histamine can be determined using ELISA or LC-MS. However these traditional methods require very expensive instrumentation and time consuming sample preparation procedures.

The *MaxSignal® Histamine Enzymatic Assay Kit* uses an enzymatic assay to detect histamine. The kit enables international and government regulatory agencies, food manufacturers and processors, as well as quality assurance organizations, to detect histamine in animal matrices in response to customer concerns about food safety. The unique features of the kit are:

- Rapid, cost-effective aqueous extraction method with high recovery
- New methanol-free extraction protocol
- High sensitivity and low detection limit (6 ppm for fresh fish/seafood)
- A very rapid (10 minutes) and robust enzyme-based assay which does not require chemical derivatization or expensive instrumentation
- High reproducibility

The kit contains histamine standards to verify assay performance and construct an assay calibration curve.

## Procedure Overview

The *MaxSignal®* Histamine Enzymatic Assay Kit measures the chemical reduction of a dye molecule using a histamine-specific enzyme. The color change caused by reduction of the dye (measured by the absorbance at 450 nm) is related to the amount of histamine present in the sample.

## Kit Contents, Storage and Shelf Life

The *MaxSignal® Histamine Enzymatic Assay Kit* has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). Store the kit at -20°C. The shelf life is 12 months when the kit is properly stored.

| Kit Contents | Amount | Storage |
|---|---|---|
| Microtiter Plate ** | 1 x 96-well Plate (8 wells x 12 strips) | -20°C |
| Histamine standards **: | | -20°C |
| 0 ppm *(white cap tube)* | 1.6 mL | |
| 1.0 ppm *(yellow cap tube)* | 1.6 mL | |
| 2.0 ppm *(orange cap tube)* | 1.6 mL | |
| 4.0 ppm *(pink cap tube)* | 1.6 mL | |
| 8.0 ppm *(purple cap tube)* | 1.6 mL | |
| 12.0 ppm *(blue cap tube)* | 1.6 mL | |
| 1,000 ppm (spiking, optional, *red cap tube)* | 1.6 mL | |
| 25X Sample Extraction Buffer | 2 x 25 mL | -20°C |
| 100X Enrichment Solution | 2 x 25 mL | -20°C |
| Master Mix ** | 6 mL | -20°C |
| Enzyme Mix | 6 mL | -20°C |

** Components with the same BIOO part No's within their expiration dates are interchangeable among BIOO kits.

## Sensitivity (Detection Limit)

| Sample Type | Detection Limit (ppm) |
|---|---|
| Meat/Seafood | 6 |
| Fish Meal | 10 |
| Milk | 40 |
| Wine | 40 |

## Specificity (Cross-Reactivity)

| Analytes | Cross-Reactivity (%) |
|---|---|
| Histamine | 100 |

## Required Materials Not Provided With the Kit

- Microtiter plate reader (450 nm)
- Tissue Mixer
- Vortex Mixer
- Methanol

## Warnings and Precautions

BIOO strongly recommends that you read the following warnings and precautions to ensure your full awareness of the techniques and other details you should pay close attention to when running the assays. Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the protocol coming with the kit. If you need further assistance, you may contact your local distributor or BIOO at techsupport2@biooscientific.com.

- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots.
- Try to maintain a laboratory temperature of 20°–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation.
- Make sure you are using only distilled or deionized water since water quality is very important.
- When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
- Add standards to plate only in the order from low concentration to high concentration, as this will minimize the risk of compromising the standard curve.
- After thawing the reagents, store reagents on ice or at 4°C (except for the extraction buffer, which can be stored at room temperature) to prevent degradation of the kit components.

# SAMPLE PREPARATION

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25°C / 68 – 77°F) or in a refrigerator before use. ☝ *Do not use glassware for extraction purposes. Histamine may adhere to glass which may affect test results.*

## Meat/Seafood

### Method I

Preparation of 1X Sample Extraction Buffer: Mix 1 volume of 25X Sample Extraction Buffer with 24 volumes of distilled water.

1. Homogenize a reasonable amount of sample (e.g. 20 g). Transfer 2 - 5 g (2 g is recommended) of the sample to a clean tube. Add 2 mL of methanol per gram of sample. Vortex mix for 2 minutes.
2. Transfer 1.5 mL of the suspended homogenate to a microcentrifuge tube. Incubate the supernatant at 70°C for 5 minutes. Vortex mix for 5 seconds.
3. Centrifuge for 5 minutes at 6,000 rpm (4,000 x g). Transfer 0.5 mL of supernatant to a new tube. (☝ *Avoid transferring any precipitate or floating solid*).
4. Add an identical volume (0.5 mL) of 1X Sample Extraction Buffer to the sample. The samples are now ready to test in the assay.

***Note:*** *Dilution factor: 6. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

### Method II

Preparation of 1X Enrichment Solution: Mix 1 volumes of 100X Enrichment Solution with 99 volumes of distilled water.

Preparation of 6X Sample Extraction Buffer: Mix 6 volumes of 25X Sample Extraction Buffer with 19 volumes of distilled water.

1. Homogenize a reasonable amount of sample (e.g. 20 g). Transfer 2 - 5 g (2 g is recommended) of the sample to a clean tube. Add 4 mL of 1X Enrichment Solution per gram of sample. (for example, add 8 ml of 1X Enrichment Solution to 2 g of homogenized fish). Vortex mix for 2 minutes.
2. Transfer 1.2 - 1.5 mL of the suspended homogenate to a microcentrifuge tube. Heat the sample at 85°C for 10 minutes. Vortex mix for 5 seconds.
3. Centrifuge for 5 minutes at 13,000 rpm (14,000 x g). Transfer 0.5 mL of supernatant to a new tube. (☝ *Avoid transferring any precipitate or floating solid*).
4. Add 0.1 mL of 6X Sample Extraction Buffer to the sample. Mix briefly. The samples are now ready to test in the assay.

***Note:*** *Dilution factor: 6. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

## Fish Meal

Preparation of 1X Sample Extraction Buffer: Mix 1 volume of 25X Sample Extraction Buffer with 24 volumes of distilled water.

1. Homogenize reasonable amount of the sample (e.g. 10 g). Take out 0.5 g of the sample to a tube; add 2.5 mL of 1X Sample Extraction Buffer. Vortex mix for 2 minutes.
2. Centrifuge for 5 minutes at 6,000 rpm (4,000 x g). Transfer 1 mL of supernatant to a new tube. (☝ *Avoid transferring any precipitate or floating solid*).
3. Incubate the supernatant at 75°C for 5 minutes. Vortex for 30 seconds.
4. Centrifuge for 5 minutes at 6,000 rpm (4,000 x g). Transfer 0.4 mL of the supernatant to a new tube. (☝ *Avoid transferring any precipitate or floating solid*). Add an identical volume (0.4 mL) of methanol to the sample.
5. Incubate the sample at 75°C for 5 minutes.
6. Centrifuge for 5 minutes at 6,000 rpm (4,000 x g). Transfer 0.4 mL of the supernatant to a new tube.

***Note:*** *Dilution factor: 12. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

## Milk

Preparation of 1X Sample Extraction Buffer: Mix 1 volume of 25X Sample Extraction Buffer with 24 volumes of distilled water.

1. Centrifuge the milk sample for 5 minutes at 6,000 rpm (4,000 x g).
2. Transfer the lower layer to a clean tube, avoiding contamination from the top layer of cream.
3. Dilute the sample 1:40 in 1X Sample Extraction Buffer.

***Note:*** *Dilution factor: 40.*

## Wine

Preparation of 1X Sample Extraction Buffer: Mix 1 volume of 25X Sample Extraction Buffer with 24 volumes of distilled water.

Dilute the sample 1:40 in 1X Sample Extraction Buffer.

***Note:*** *Dilution factor: 40.*

*Preparation protocols for samples other than above can be made available upon request. Please contact your local distributor or write us at foodfeedsafety@biooscientific.com.*

# HISTAMINE DETECTION TEST PROTOCOL

## Reagent Preparation

IMPORTANT: All reagents should be warm up to room temperature (20 – 25°C / 68 – 77°F); Make sure you read "Warnings and Precautions" section. Solutions should be prepared just prior to Enzymatic Assay. All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended. The thawed reagents can be stored at 4°C for as long as 2 weeks; for a longer time period storage, the reagents should be put in a freezer.

1. Preparation of Reaction Mix
   Thaw out Master Mix and Enzyme Mix and warm to room temperature (20 – 25°C / 68 – 77°F). Invert tube three times to mix thoroughly. In a clean tube, mix Master Mix and Enzyme Mix (1:1, V/V). Use the following table to calculate the required volume for the Reaction Mix:

| Reaction Mix | | Volume per Reaction | 24 Reactions |
|---|---|---|---|
| Master Mix | Enzyme Mix | 100 µL | 2.4 mL |
| 1.2 mL | 1.2 mL | | |

   Note: Store the complete reaction mix on ice or at 4°C until use to prevent background signal.

2. Preparation of 67% Methanol
   Dilute 100% methanol to 67% using $dH_2O$.

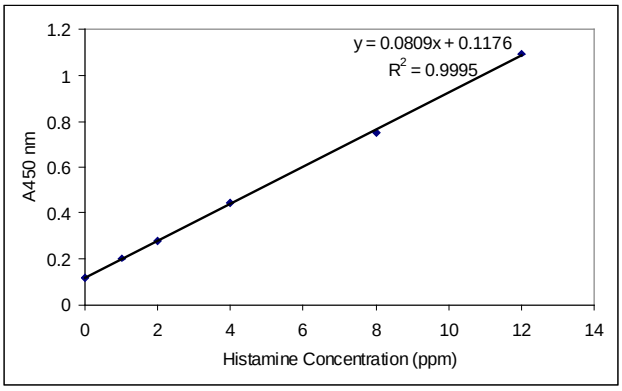
3. Standards
   Thaw out the 6 tubes containing Histamine Standards. Warm to room temperature (20 – 25°C / 68 – 77°F).

## Enzymatic Testing Protocol

1. Samples: Add 100 µL of each sample in duplicate to wells.
2. Histamine Standards: Add 50 µL of 67% methanol plus 50 µL of each standard (in duplicate) to wells of the 96-well microtiter plate.
   (Note: If you are using Method II of the Meat/Seafood Extraction Protocol, substitute 50 µL of 1X Enrichment Solution instead of 50 µL 67% methanol).
3. Add 100 µL of the Reaction Mix to each well.
4. Incubate for 10 minutes at room temperature.
5. Measure absorbance of each well at 450 nm in a plate reader.

## Histamine Concentration Calculation

A standard curve can be constructed by plotting the mean corrected absorbance obtained from each reference standard against its concentration in ppm.




Calculate the slope and the y-intercept for the line which fits the standard curve data.

The histamine concentration in the well can be described by the equation:
Concentration = (mean absorbance – y-intercept)/slope
Histamine concentration in starting sample = concentration x dilution factor (e.g. 6 for seafood samples).

Use the mean absorbance values for each sample to determine the corresponding concentration of the tested drug in ppm from the standard curve. The *MaxSignal® Enzymatic Assay Analysis Program in Excel* is available upon request to evaluate the test results. Please contact your local distributor or techsupport2@biooscientific.com for further information.

## RELATED PRODUCTS

| Product | Catalog Number |
|---|---|
| HistaStrip™ Test Kit | 1100-01 |
| MaxSignal® Histamine Enzymatic Assay for Fish Sauce | 1052-01 |

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