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MAX SIGNAL®

Histamine Enzymatic Assay Kit Manual

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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 such as tuna, mahi mahi, and swordfish or in cheese. 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.

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 extraction method with high recovery.
- High sensitivity and low detection limit (4 ppm for fresh fish/seafood/cheese).
- A very rapid (10 minutes) and robust enzyme-based assay which does not require chemical derivatization or expensive instrumentation.
- High reproducibility.
- No heating, centrifugation, washing or incubation steps.

The kit contains both a positive and a negative control to verify assay performance.

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

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)	Room temp
Histamine standards:		-20°C
Negative control *(white cap tube)*	0.8 mL	
0.35 ppm *(yellow cap tube)*	0.8 mL	
0.7 ppm *(orange cap tube)*	0.8 mL	
1.4 ppm *(pink cap tube)*	0.8 mL	
2.8 ppm *(purple cap tube)*	0.8 mL	
7 ppm *(blue cap tube)*	0.8 mL	
1,000 ppm (spiking, optional, *red cap tube)*	0.8 mL	
Master Mix	12 mL	-20°C
Enzyme Mix	12 mL	-20°C

Sensitivity (Detection Limit)

Sample Type	Detection Limit (ppm)
Seafood	4
Cheese	4

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
- Microcentrifuge and microcentrifuge tubes (Optional)
- Filter paper, such as Whatman #4 (Optional)

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*.

Seafood

1. Homogenize a small portion of sample (>10 g). Transfer a reasonable amount of sample (2 - 5 g is recommended but 1 g is also acceptable) to a clean tube. Add 9 mL of methanol per gram of sample to each tube.
2. Vortex mix for 1 minute. Allow sample to settle for 5 minutes.
3. Repeat step #2. If floating particles are visible in the liquid, remove them by centrifugation or filtration. The clear methanol supernatant is now ready to be tested.

Note: *Dilution factor: 10. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

Alternative Rapid Method for Seafood using a Microcentrifuge

1. Homogenize a small portion of sample (>10 g). Transfer a reasonable amount of sample (2 g is recommended but 1 g is also acceptable) to a clean tube. Add 9 mL of methanol per gram of sample to each tube.
2. Vortex mix for 4 minutes. Transfer 1 mL of sample to a microcentrifuge tube and centrifuge at 10,000 rpm for 1 minute. The clear methanol supernatant is now ready to be tested.

Note: *Dilution factor: 10. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

Alternative Rapid Method for Seafood using a Filter

1. Homogenize a small portion of sample (>10 g). Transfer a reasonable amount of sample (2 g is recommended but 1 g is also acceptable) to a clean tube. Add 9 mL of methanol per gram of sample to each tube.
2. Vortex mix for 4 minutes. Filter the sample using filter paper (such as Whatman #4) into a clean tube. The clear methanol supernatant is now ready to be tested.

Note: *Dilution factor: 10. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

Cheese

1. Homogenize a small portion of sample (>5 g). Transfer a reasonable amount of sample (2 g is recommended but 1 g is also acceptable) to a clean tube. Add 9 mL of 50% methanol per gram of sample to each tube.
2. Vortex mix for 1 minute. Allow sample to settle for 5 minutes.
3. Repeat step #2.

4. Transfer 1 mL of sample to a microcentrifuge tube and centrifuge at 10,000 rpm for 1 minute. The clear methanol supernatant is now ready to be tested.

   Note: *Dilution factor: 10. If possible, a known negative sample could be prepared in parallel; subtract the negative sample from the test sample results.*

   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). 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 Composition (per reaction)		Volume per Reaction (Total)	24 Reactions	96 Reactions
Master Mix	Enzyme Mix			
100 µL	100 µL	200 µL	4.8 mL	19.2 mL

Note: If you are not using the Reaction Mix immediately, store the complete reaction mix on ice or at 4°C until use to prevent background signal.

2. **Standards**
   Warm up the 6 tubes containing Histamine Standards to room temperature (20 – 25°C).

Enzymatic Testing Protocol- Seafood

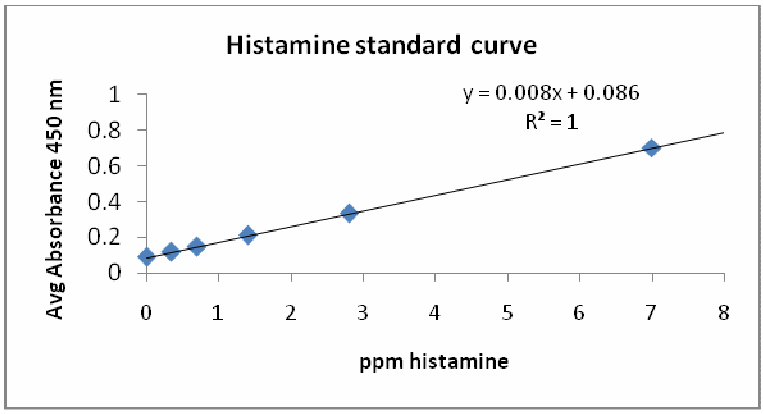
1. Add 100 µL of each sample or standard (in duplicate) to Microtiter Plate wells.
   (*Add standards to plate only in the order from low concentration to high concentration*).
2. Add 200 µL of the Reaction Mix to each well.
3. Incubate for 10 minutes at room temperature.
4. Measure absorbance of each well at 450 nm in a plate reader.

Enzymatic Testing Protocol- Cheese

1. Add 100 µL of each sample or standard (in duplicate) to microfuge tube.
   (✍ *Add standards to plate only in the order from low concentration to high concentration*).
2. Add 200 µL of the Reaction Mix to each well. Mix briefly.
3. Incubate for 5 minutes at room temperature.
4. Microfuge 1 minute at 10,000 rpm. Transfer 250 µL of supernatant to Microtiter Plate wells.
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 (= 10 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. A special program with Excel functionality, *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.

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