AlerTox® ELISA Histamine Kit

A competitive ELISA test for the quantitative detection of histamine in food products like fish and wine

Product no. KIT3065 (96 reactions)

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1. Introduction

Histamine is a biogenic amine that is formed by enzymatical decarboxylation of the amino acid histidine. This occurs in mast cells and basophilic white cells bound to heparin. In a type I allergic response, endogenic histamine from mast cells and basophilic leucocytes are released after antigen binding to membrane-associated IgE, resulting in typical allergic reactions. However, histamine can also enter the human body via ingested food and drink and can thus cause pseudo-allergic food intolerances.

Food intolerances, which are caused by increased histamine concentrations, are clinically characterized by rash, diarrhea, vomiting, nausea, itching, headache and asthma. The extent of the reaction is dependent on the ingested amount of histamine. Toxic histamine concentrations may arise from inappropriate handling or a disrupted cold chain. They can cause the so-called scombroid reaction, which appears after bacterial degradation of protein-rich food, especially fish from the Scombridae family. The US Food and Drug Administration has established an action level of 50 ppm for histamine in fish. According to EU Regulation 2073/2005 and further amendments, the limit for histamine in fish or fish products is 100 ppm. This limit is raised to 200 ppm if the fish or fish products have undergone enzymatic maturation in brine.

Thus, histamine concentrations in fish and fish products must be monitored. The AlerTox® ELISA Histamine Kit is a sensitive detection system, capable of rapid quantification of histamine concentrations in fish and wine.

Note: Read this manual carefully before starting the test. The test must be performed by thoroughly trained staff.

1.1 Test Sensitivity and Specificity

The AlerTox ELISA Histamine Kit provides reagents for the quantitative analysis of histamine in fresh and processed food products, such as fish and wine. The limit of detection (LOD) is between 0.3 – 0.7 ppm of histamine, the limit of quantification (LOQ) is 2.0 ppm and the detection is quantitative between 2.0 and 72 ppm (see *Section 6.1, Summary of Specifications*, for more details). The cross-reactivity with other food matrices is shown in the following table:

Cross-Reactive Matrix	Percent Cross-Reactivity (%)
Histamine	100
1-Methylhistamine	5
N-Acetylhistamine	0.02

See *Section 6.2, Recovery* and *Section 6.3, Non-Cross Reactivity*, for additional data.

Important: This histamine kit uses a competitive ELISA for detection. Therefore, the sample preparation and ELISA protocols differ from those of other AlerTox ELISA Kits, which are based on sandwich ELISA testing.

Important: Do not modify the protocol with respect to the timing, temperatures, plate washing, pipetting volumes, types of buffers or pH values of the buffers. Any of these protocol modifications will cancel the validation of the test system.

1.2 Test Principle

The AlerTox ELISA Histamine test is based on the principle of a quantitative competitive ELISA. The histamine concentration is indirectly proportional to the color intensity of the test sample. Here is a brief overview of the competitive ELISA test:

1. A histamine conjugate is bound on the surface of a microtiter plate. Standards containing derivatized histamine or test samples and an antibody directed against histamine (primary antibody) are added to wells of the microtiter plate. Immobilized and free histamine compete for the antibody binding sites. After a

5-minute incubation at room temperature, the wells are washed with washing solution to remove unbound material.

2. A peroxidase-conjugated secondary antibody directed against the histamine antibody is added into the wells, and after another 5-minute incubation, the plate is washed again.
3. A substrate solution is added and incubated for 5 minutes, resulting in the development of a blue color. The addition of a stop solution inhibits the color development, and the color turns yellow. The yellow color is measured photometrically at 450 nm (OD_{450}).

2. Materials and Storage

2.1 Materials Supplied in the Kit

The kit contains sufficient reagents for 96 determinations (including standards).

Item	Description	96 wells
1	Breakable strips of 8 wells, each coated with histamine conjugates.	12 strips
2	6 AlerTox Histamine Standards, concentrations: 0 – 2.0 – 6.0 – 12.0 – 24.0 – 72.0 ppm.	6 x 4 mL
3	Reaction Solution, containing 1,4-benzoquinone	1 x 3 mL
4	Neutralizing Solution	1 x 15 mL
5	Anti-Histamine Antibody (primary antibody, blue solution)	1 x 6 mL
6	Conjugate Solution, Anti-rabbit-IgG-horseradish peroxidase (HRP) (secondary antibody, red solution)	1 x 15 mL
7	Substrate Solution, containing trimethylbenzene (TMB).	1 x 15 mL
8	Stop Solution, containing sulfuric acid (H_2SO_4).	1 x 15 mL
9	Sample Dilution Buffer (red solution)	2 x 60 mL
10	10X Washing Solution	1 x 60 mL

2.2 Storage Conditions and Stability

- All kit components should be kept at 2 to 8 °C (36 to 46 °F) in the dark. DO NOT FREEZE.
- Return all reagents to 2 to 8 °C (36 to 46 °F) in the dark immediately after use.
- The diluted Washing Solution (1X) can be used for 4 weeks when stored at 2 to 8 °C (36 to 46 °F).

  Important: If needed, redissolve precipitants by warming the 10X Washing Solution at 37 °C (99 °F) for 15 minutes before dilution. Do not use the buffer if the precipitant does not redissolve.
- The Sample Extracts are stable for at least 24 hours at 2 to 8 °C (36 to 46 °F) or longer when frozen.

2.3 Material Required but Not Provided

- Multi-channel pipettor: 50 – 200 µL
- Sterile pipette tips
- Pipettors: 10 – 100 µL, 100 – 1,000 µL
- Water bath, adjustable to 60 °C (140 °F)
- 15 – 100 mL containers for the extractions
- Stomacher, homogenizer, mill, mortar or blender
- ELISA Plate Reader with filter (450 nm) (Product No. MCH3005, or similar)
- Centrifuge
- Double distilled water
- Vortex mixer
- Paper towels

2.4 Optional Materials/Equipment

- Homogenizer for sample extraction
- Repeating pipettor to minimize assay drift
- *Recommended*: An ELISA plate washer system to reduce the washing time and improve consistency

3. Test Procedure

3.1 Reagent Preparation

We advise preparing reagents immediately before use and only preparing the amount necessary for the number of samples plus the 6 standards. Duplicate measurements of each sample and standard are recommended based on good laboratory practices (GLP) and quality control requirements.

Important: All reagents must be at room temperature (15 to 25 °C, 59 to 77 °F) at the time of use.

3.1.1 Prepare Washing Buffer

Dilute the 10X Washing Buffer 1:10 with distilled water to create the 1X solution.

Important: If needed, redissolve precipitants by warming the 10X Washing Buffer at 37 °C (99 °F) for 15 minutes before dilution. Do not use the buffer if the precipitant does not redissolve.

Note: You will need approximately 1.9 mL of 1X Washing Buffer per well.

3.1.2 Set Up ELISA Plate

To prepare the ELISA plate, open the foil bag, only remove the number of strips required to run the tests (samples plus the 6 standards, all in duplicate) and put the strips into the frame.

Notes:

- When opening the foil bag for the first time, be careful not to cut the ziplock off the bag.
- Unused wells must be stored in the foil bag with the drying agent at 2 to 8 °C (36 to 46 °F). Ensure the ziplock on the foil bag is sealed tightly.

3.*1.3 Prepare Standards (Derivatization)*

1. Transfer 500 µL of each standard into separate tubes and add 25 µL of Reaction Solution to each standard.
2. Mix thoroughly and incubate for 1 min.
3. Add 100 µL of Neutralizing Solution to the activated standard.
4. Mix thoroughly and incubate for 1 min.

   Note: These standards are now derivatized and ready for ELISA testing.

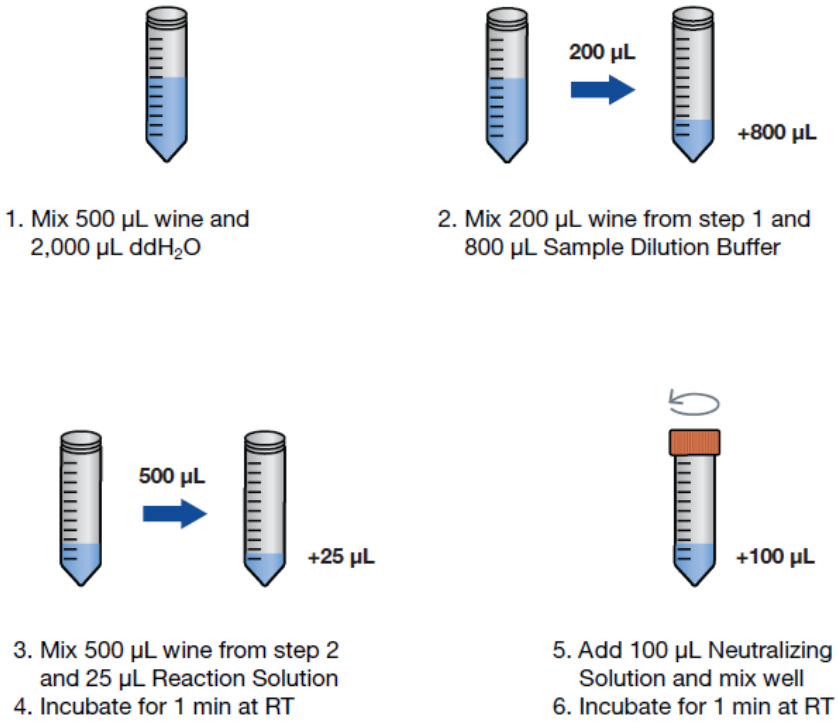
3.2 Sample Preparation (Derivatization)

3.2.1 Wine

1. Mix 500 µL of wine with 2,000 µL of double distilled water (ddH_2O).
2. Transfer 200 µL of this mixture (Step 1) into a new tube containing 800 µL of Sample Dilution Buffer.
3. Transfer 500 µL of the diluted wine sample (Step 2) into a new tube containing 25 µL of Reaction Solution.
4. Mix thoroughly and incubate for 1 min at room temperature (15 to 25 °C, 59 to 77 °C).
5. Add 100 µL of Neutralizing Solution to the activated sample (Step 4).
6. Mix thoroughly and incubate for 1 min at room temperature.

   Note: This sample is now derivatized and ready for ELISA testing.

3.2.2 Workflow Overview (Wine)



1. Mix 500 µL wine and 2,000 µL ddH_2O
2. Mix 200 µL wine from step 1 and 800 µL Sample Dilution Buffer
3. Mix 500 µL wine from step 2 and 25 µL Reaction Solution
4. Incubate for 1 min at RT
5. Add 100 µL Neutralizing Solution and mix well
6. Incubate for 1 min at RT

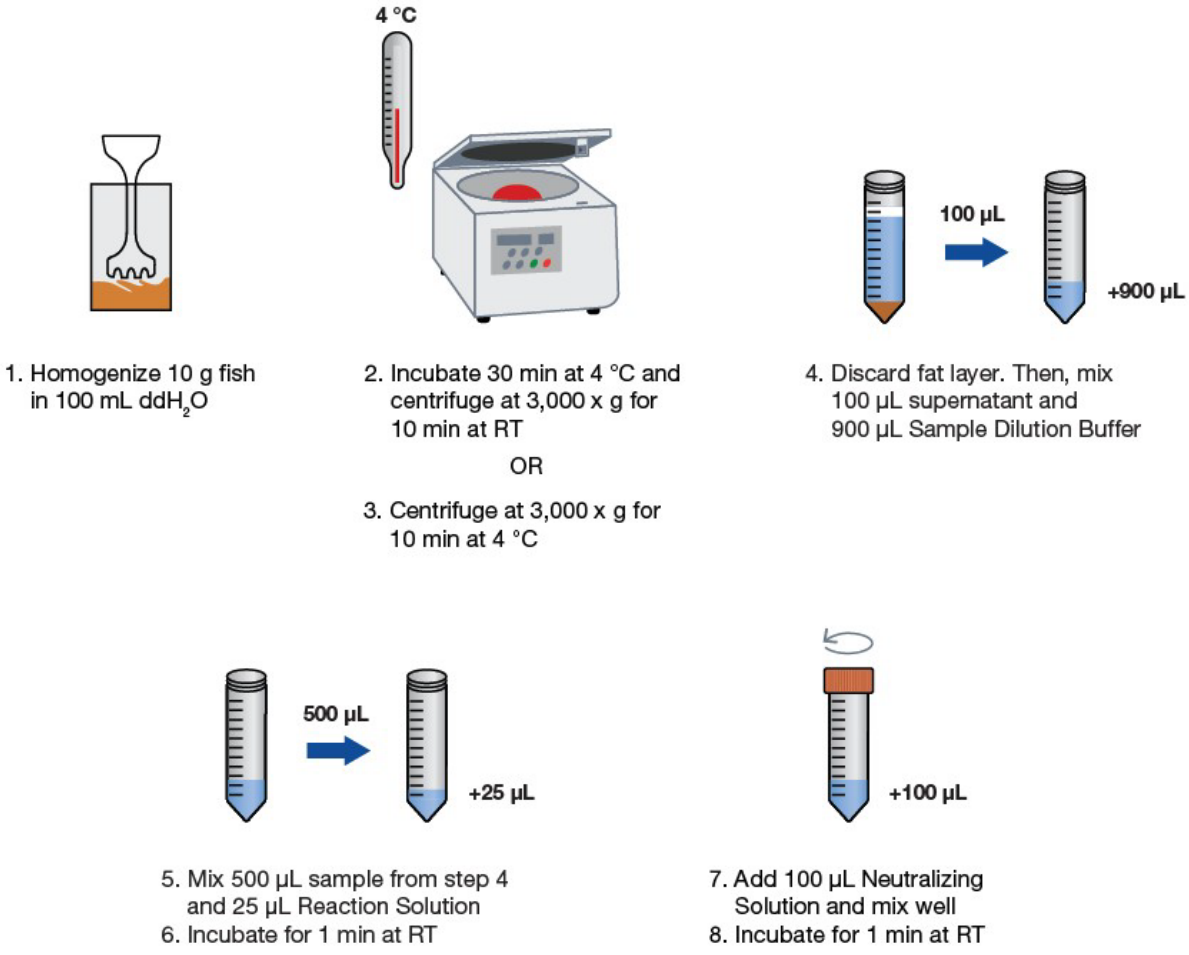
3.2.3 Fish

1. Homogenize 10 g of fish sample in 100 mL of double distilled water (ddH_2O).
2. Incubate for 30 minutes at 4°C (39 °F).

   Note: This step can be skipped if step 3 is performed with a centrifuge at 4°C (39 °F).
3. Centrifuge at a minimum of 3,000 x *g* for at least 10 minutes.
4. Discard the upper fat layer and transfer 100 µL of the supernatant into a new tube containing 900 µL of Sample Dilution Buffer.
5. Transfer 500 µL of the diluted fish sample (Step 4) into a new tube containing 25 µL of Reaction Solution.
6. Mix thoroughly and incubate for 1 min at room temperature (15 to 25 °C, 59 to 77 °C).
7. Add 100 µL of Neutralizing Solution to the activated sample (Step 6).
8. Mix thoroughly and incubate for 1 min at room temperature.

   Note: This derivatized sample is now ready for ELISA testing.

3.2.4 Workflow Overview (Fish)



1. Homogenize 10 g fish in 100 mL ddH_2O
2. Incubate 30 min at 4 °C and centrifuge at 3,000 x g for 10 min at RT

   OR

   3. Centrifuge at 3,000 x g for 10 min at 4 °C
4. Discard fat layer. Then, mix 100 µL supernatant and 900 µL Sample Dilution Buffer
5. Mix 500 µL sample from step 4 and 25 µL Reaction Solution
6. Incubate for 1 min at RT
7. Add 100 µL Neutralizing Solution and mix well
8. Incubate for 1 min at RT

3.3 ELISA Procedure

Important: The most critical points of the ELISA procedure are the *temperature*, *timing* and *plate washing*. Insufficient washing will result in poor precision and false results.

Note: For higher reproducibility, we recommend using a well-maintained, automated plate washer in steps 4 and 7 below.

1. Add 100 µL derivatized standards or sample extracts in duplicate into the appropriate wells of the microtiter plate.

   Note: See *Section 7, Example Assay Layout*. If you have a large number of samples, pipette one set of standards before the samples and the duplicate set of standards after the samples and use the arithmetic mean values for calculations.

2. Add 50 µL of Anti-Histamine Antibody into each well.
3. Incubate for 5 minutes at room temperature (15 to 25 °C, 59 to 77 °F).
4. Wash the plate three times with 300 µL of 1X Washing Buffer per well.

   Note: At the end of the automated washing or between each manual wash, invert the plates and strike the plate against clean, dry paper towels to empty the wells and remove residual liquid.

5. Add 100 µL of Conjugate Solution into each well.
6. Incubate for 5 minutes at room temperature (15 to 25 °C, 59 to 77 °F).
7. Wash the plate as described in step 4.
8. Add 100 µL of Substrate Solution into each well.
9. Allow the reaction to develop in the dark (the chromogen is light sensitive) for 5 minutes at room temperature (15 to 25 °C, 59 to 77 °F).
10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well.

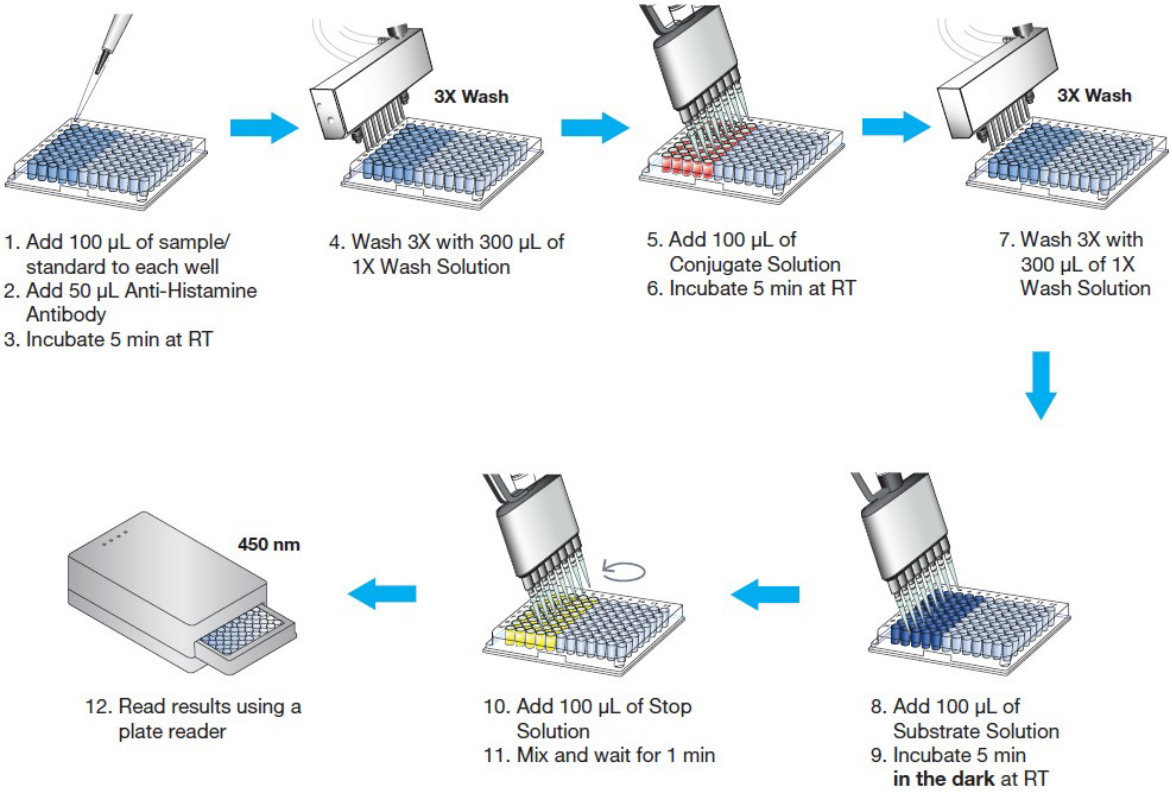
    Note: Wells containing blue color will turn yellow in the presence of derivatized histamine.

11. Gently shake the plate by hand and wait for 1 minute.
12. To measure results, use an ELISA plate reader with a 450 nm filter ($OD_{450\ nm}$), following the instrument manufacturer's instructions.

    Note: Measure the color change within 30 minutes.

    Important: If any sample results fall outside the range of the derivatized histamine standard curve, do not extrapolate the data. Instead, dilute the sample extract further with 1X Extraction & Sample Dilution Buffer and repeat the ELISA test using this diluted sample extract and standards, in duplicate.

3.3.1 Workflow Overview



4. Results Calculations

The standards are prepared for a direct determination of histamine concentration in the sample. The dilution of samples in the extraction process, as described in the sample preparation procedures, is already taken into consideration when calculating levels. However, results must account for any additional dilution (e.g., due to high sample concentration) and for wine samples (see Step 4 below).

1. Calculate the mean OD value ($OD_{450\ nm}$) for each set of reference standards and samples.
2. To create the standard curve, plot the mean OD values of the reference standards on the y-axis versus the concentration of histamine in ppm on the x-axis.
3. Using the mean optical density (OD) value for each sample, determine the corresponding concentration of histamine in ppm from the standard curve.
4. For wine samples, multiply the results by 0.25 to obtain the concentration of histamine in the original sample.

5. General Precautions

- If your skin comes in contact with toxic or irritating substances, rinse the affected area with plenty of water and seek medical attention if needed. Please refer to the SDS, available at www.hygiena.com/SDS.
  - The Reaction Solution contains 1,4-benzoquinone. If this solution contacts skin, rinse the affected area with plenty of water. Please refer to the SDS.
  - The Substrate Solution contains TMB, which is highly toxic if inhaled, ingested or contacts skin. Please refer to the SDS.
  - The Stop Solution contains H_2SO_4, which is corrosive. Please refer to the SDS.

- Handle the test kit in accordance with GLP.
  - Do not use reagents beyond the expiration date of the kit.
  - Handle all solutions with gloves.
  - During the sample extraction, avoid cross-contamination.
  - Devices, such as a blender, must be cleaned after each sample preparation.
  - Use sterile pipette tips.
  - Do not exchange reagent vial caps.
  - Do not interchange reagents between kits of different lot numbers.
- Do not alter reagents. Doing so can cause inaccurate results.
- All reagents must be equilibrated at room temperature (15 to 25 °C, 59 to 77 °F) before use.
- Do not use solutions if they become cloudy or precipitate. The only exception is the 10X Washing Buffer, which may have crystalline precipitants that must be completely dissolved before use (see Section 2.2).
- Substrate Solution is light sensitive. Avoid exposure to direct light and store in the dark.
- Use only distilled water for the dilution of concentrated buffers.
- Do not allow wells to dry completely.
- Avoid incubating microtiter plates on cold work benches.

6. Additional Information

6.1 Summary of Specifications

Specification	AlerTox ELISA Histamine	
Target	Histamine	
Limit of Detection (LOD)	0.3 – 0.7 ppm	
Limit of Quantification (LOQ)	2 ppm	
Standard Range	0.0 – 72 ppm	
Quantification Range	2.0 – 72 ppm	
Calculation Factors	Wine	Multiply the results by 0.25
	Fish	No additional calculations are needed

For lot-specific assay data and acceptance/rejection criteria for measured values, see the Certificate of Analysis (www.hygiena.com/COA).

6.2 Recovery

Matrix*	Recovery (%)
Codfish	102
Plaice	95
Salmon	84
Trout	96
Tuna	99
Wine (red)	97

* Tested in typical matrices.

6.3 Non-Cross Reactivity

Of the matrices that were tested, the following were found to be non-cross-reactive with the AlerTox ELISA Histamine Kit:

Non-Cross-Reactive Matrices
Histidine
Serotonin

7. Example Assay Layout

S0: Zero Standard (without antigen). The mean value = B_0.

S1 – S5: Standards. The mean value is B.

SP: Samples. The mean value is B.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**S0**	**S0**	SP3	SP3	SP11	SP11						
B	**S1**	**S1**	SP4	SP4	SP12	SP12						
C	**S2**	**S2**	SP5	SP5	Etc.	Etc.						
D	**S3**	**S3**	SP6	SP6	Etc.	Etc.						
E	**S4**	**S4**	SP7	SP7	Etc.	Etc.						
F	**S5**	**S5**	SP8	SP8	Etc.	Etc.						
G	SP1	SP1	SP9	SP9	Etc.	Etc.						
H	SP2	SP2	SP10	SP10	Etc.	Etc.						

8. Disclaimer

These products are made from high-quality raw materials. No warranty of any kind is made, either expressed or implied, as to their suitability other than to measure the target antigen content when used exactly in accordance with these instructions, except regarding the quality of these materials.

Use of the kit for any other purpose is outside its intended use. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.

9. Contact Information

For more information, visit www.hygiena.com/contact. For technical support, visit www.hygiena.com/support.

10. Change Index

INS3065 REVA, February 2022
Initial instructions.

INS-KIT3065-001-REVA, January 2025
Minor edits in Section 2.1. Added graphic workflows. Updated layout and document ID number.

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