Histamine ELISA Kit

Catalog Number KA1888

96 assays

Version: 05

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for the quantitative determination of Histamine in stool.

Principle of the Assay

Histamine is quantitatively acylated.
The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. Analyte concentrations of acylated standards, controls and samples and solid phase bound analyte concentrations compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

General Information

Materials Supplied

List of component

Component	Description	Amount
Reaction Plate	ready for use	96 wells
Wash buffer Concentrate	concentrate, dilute content with dist. water to a final volume of 1000 mL	20 mL
Substrate	ready for use, containing a solution of tetramethylbenzidine (TMB)	12 mL
Stop Solution	ready for use, containing 0.25 M H_2SO_4.	12 mL
Histamine Microtiter Strips	12 strips, 8 wells each, break apart, precoated	96 (8x12) wells
Standard A	ready for use	4 mL
Standard B	ready for use	4 mL
Standard C	ready for use	4 mL
Standard D	ready for use	4 mL
Standard E	ready for use	4 mL
Standard F	ready for use	4 mL
Control 1	ready for use	4 mL
Control 2	ready for use	4 mL
Histamine Antiserum	from goat, ready for use	12 mL
Acylation Buffer	ready for use	12 mL x 2
Acylation Reagent	ready for use	1.5 ml x 2
Enzyme Conjugate	ready for use, anti-goat IgG conjugated with peroxidase	12 ml

Storage Instruction

Store the reagents at 2-8℃ until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

Materials Required but Not Supplied

- ✓ Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1,000 µL)
- ✓ Microtiter plate washing device
- ✓ sample tubes with a volume > 5ml
- ✓ Eppendorf-tube or similar centrifugation device
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Centrifuge capable of at least 3.000 x g

- ✓ Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- ✓ Absorbent material (paper towel)
- ✓ Distilled water
- ✓ Vortex mixer

Precautions for Use

- ✓ Reliability of the test results

  In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions. It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

- ✓ Complaints

  In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

- ✓ Warranty

  This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

- ✓ Disposal

  Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

- ✓ Interference

  Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

- ✓ Precautions

  Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation. All reagents of this test kit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

Assay Protocol

Reagent Preparation

- ✓ Wash Buffer

  Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL. Storage: up to 6 months 2-8°C.

- ✓ Acylation Reagent

  The Acylation Reagent has a freezing point of 18.5°C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternative the Acylation Reagent can be stored at room temperature (20-25°C) separate from the other kit components.

Sample Preparation

- ✓ Stabilized samples can be stored one week at RT (20-25°C) or up to 6 months at 2-8°C.
- ✓ Diluent stool samples as 1:300. Assay data must be corrected accordingly.

Assay Procedure

Allow reagents and samples to reach room temperature. Duplicate measurements are recommended.

- ✓ Preparation and acylation

1. Pipette 100 µL of standards, 100 µL of controls and samples into the respective wells of the Reaction Plate.
2. Add 25 µL of Acylation Reagent to all wells.
3. Pipette 200 µL of Acylation Buffer into all wells.
4. Shake Reaction Plate shortly by hand and incubate 15 min at RT (20-25°C).
   Note: Take 25 µL for the ELISA

- ✓ Histamine ELISA

1. Pipette 25 µL of the acylated standards, controls and samples into the wells of the Histamine Microtiter Strips.
2. Pipette 100 µL of the Histamine Antiserum into all wells.
3. Incubate 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
   Alternatively without shaking, shake Histamine Microtiter Strips shortly by hand and incubate for 40 min at RT (20-25°C).
4. Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL Wash

buffer. Blot dry by tapping the inverted plate on absorbent material.

5. Pipette 100 µL of the Enzyme Conjugate into all wells.
6. Incubate for 10 min at RT (20-25°C) on a shaker (approx. 600 rpm).
   Alternatively without shaking, incubate for 20 min at RT (20-25°C).
7. Discard or aspirate the contents of the wells and wash each well 3 times thoroughly with 300 µL Wash buffer. Blot dry by tapping the inverted plate on absorbent material.
8. Pipette 100 µL of the Substrate into all wells.
9. Incubate for 15 ± 2 min at RT (20-25°C) on a shaker (approx. 600 rpm).
   Alternatively without shaking, incubate for 15 ± 2 at RT (20-25°C).
   Note: Avoid exposure to direct sun light!
10. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
11. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

Data Analysis

Calculation of Results

The calibration curve from which the concentrations in the samples can be taken is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis).

Use non-linear regression for curve fitting (e.g. spline, 4-parameter, akima).

	Concentration of the standards					
Standard	A	B	C	D	E	F
Histamin (ng/ml)*	0	0.5	1.5	5	15	50
Conversion:	Histamine (ng/mL) x 9 = Histamine (nmol/L) Histamine (ng/g) x 9 = Histamine (nmol/kg)					

*Controls: The concentrations of the Controls 1 & 2 can be read directly from the standard curve.

*Stool sample: The read concentrations of the stool samples have to be multiplied by 300.

- ✓ Quality control

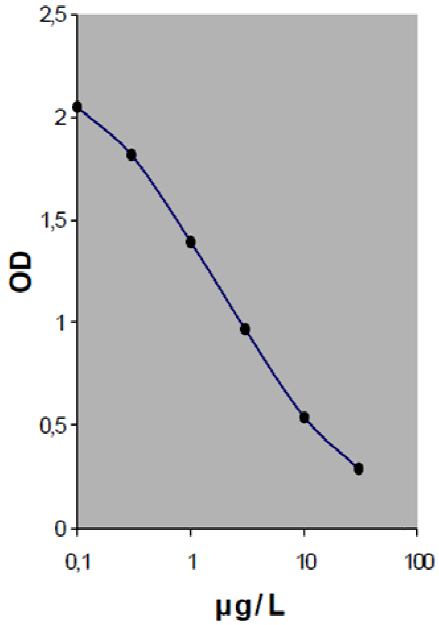
  It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

- ✓ Calibration

  The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25℃.

Note: In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

✓ Typical calibration curves (Example. Do not use for calculation!)



Performance Characteristics

✓ Expected Reference Values

Stool	Histamine
	< 600 ng/g

✓ Analytical Specificity (Cross Reactivity)

Substance	Cross Reactivity (%)Histamine
Histamine	100
3-Methyl-Histamine	0.1
Tyramine	0.01
L-Phenylalanine	< 0.001
L-Histidine	< 0.001
L-Tyrosine	< 0.001
Tryptamine	< 0.001
5-Hydroxy-Indole-Acetic Acid	< 0.001
Serotonin	< 0.001

✓ Analytical Sensitivity (Limit of Detection)

Histamine: 75 ng/g

Mean signal (Zero-Standard) - 2SD

✓ Precision

Inter-Assay Variation, n = 13			Intra-Assay Variation, n = 39		
Sample	Mean ± SD (ng/g)	CV (%)	Sample	Mean ± SD (ng/g)	CV (%)
1	868 ± 69	8	1	610 ± 73	12
2	2877 ± 161	5.6	2	4690 ± 310	6.6

✓ Linearity

Histamine	Range (ng/g)	Range (%)	Mean (%)
	5170 - 45	85 - 106	100

✓ Recovery

Histamine	Serial dilution	Range (%)	Mean (%)
	1:16	92 - 120	103

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								