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Instructions for use

Histamine Research ELISA ™

REF **IB89142R**

2°C–8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Histamine in different animal species and biological fluids.

During the sample preparation Histamine is quantitatively acylated. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After 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 standard concentrations.

2. Procedural Cautions, Guidelines and Warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) The principles of Good Laboratory Practice (GLP) have to be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
(4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A standard curve must be established for each run.
(12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(13) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(14) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(15) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(16) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

BA D-0090 FOILS **Adhesive Foil** - Ready to use
Content: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x
Content: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 ml/vial, light purple cap

BA D-0024 REAC-PLATE **Reaction Plate** - Ready to use
Content: 1 x 96 well plate, empty in a resealable pouch

BA E-1040 CONJUGATE **Enzyme Conjugate** - Ready to use
Content: Donkey anti-goat immunoglobulins conjugated with peroxidase
Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use
Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use
Content: 0.25 M sulfuric acid
Volume: 1 x 12 ml/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

BA E-1031 MTP HIS **Histamine Microtiter Strips** - Ready to use
Content: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant.

BA E-1010 HIS-AS **Histamine Antiserum** - Ready to use
Content: Goat Anti- Histamine antibody, blue coloured
Volume: 1 x 12 ml/vial, blue cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration ng/ml	Concentration nmol/l	Volume/ Vial
BA E-1001	STANDARD A	white	0	0	4 ml
BA E-1002	STANDARD B	light yellow	0.5	4.5	4 ml
BA E-1003	STANDARD C	orange	1.5	13.5	4 ml
BA E-1004	STANDARD D	dark blue	5	45	4 ml
BA E-1005	STANDARD E	light grey	15	135	4 ml
BA E-1006	STANDARD F	black	50	450	4 ml
BA E-1051	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-1052	CONTROL 2	dark red			4 ml

Conversion: Histamine (ng/ml) x 9 = Histamine (nmol/l)
Content: Acidic buffer spiked with defined quantity of Histamine

BA E-1011 ACYL-BUFF **Acylation Buffer** - Ready to use
Content: TRIS-buffer containing a non-mercury preservative
Volume: 1 x 4 ml/vial, pink cap

BA E-1012 ACYL-REAG **Acylation Reagent** - Lyophilized

Content: Lyophilized acylation reagent

Volume: 2 vials

BA E-0085 ACYL-SOLV **Acylation Solvent** - Ready to use

Content: Organic solvent

Volume: 1 x 5 ml/vial, brown cap

Hazards identification:

H225 Highly flammable liquid and vapour.

BA E-0041 DILUENT **Diluent** - Ready to use

Content: Acidic buffer with non-mercury preservatives

Volume: 1 x 22 ml/vial, white cap

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 300 µl; 2 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

5. Sample collection and storage

The kit was validated for EDTA –plasma from different animal species. In principle other sample types than plasma are also suitable but have to be tested in advance. For more details please contact your local supplier or the manufacturer directly.

In general haemolytic and lipemic samples should not be used with this assay.
Storage of plasma samples: up to 6 hours at 2 – 8 °C; for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.

6. Test procedure

The following protocol for rat plasma samples should be used as a guideline and is suitable for animal species where high Histamine concentrations are expected. In such cases, the samples have to be prediluted with the Diluent (BA E-0041). In cases, where low concentrations are expected, no sample predilution will be necessary.
The following concentrations were detected with the Histamine Research ELISA in different animal species:

Animal species	Concentration (ng/ml)
Mouse	22.9
Rat	20
Cat	1.1
Dog	0.3
Horse	0.6

Allow all reagents and samples to reach room temperature and and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Varying incubation times will have similar influences on the absorption. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

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

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.
Storage: 1 month at 2 – 8 °C

Acylation Solution
Reconstitute each vial of the Acylation Reagent (BA E-1012) with 2 ml Acylation Solvent (BA E-0085). Please make sure that it is completely dissolved before use.
If more than 2 ml are needed, pool the content of the individual vials and mix thoroughly.
Storage: 1 month at 2 – 8 °C

Histamine Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Sample predilution

1.	Pipette **10 µl** of the sample into an Eppendorf tube or similar device.
2.	Add **200 µl** of **Diluent**.
3.	Vortex for **1 min** at **RT** (20 – 25 °C).
4.	**25 µl of the prediluted sample are needed for the subsequent acylation step.**

6.3 Sample preparation and acylation

1.	Pipette **25 µl** of **standards**, **controls** and **plasma samples** into the respective wells of the **Reaction Plate.**
2.	Add **25 µl** of **Acylation Buffer** to all wells.
3.	Add **25 µl** of **Acylation Solution** (refer to 6.1) to all wells.
4.	Incubate for **45 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
5.	Add **100 µl** of **water** (deionized, distilled, or ultra-pure) to all wells.
6.	Incubate for **15 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	**Take 25 µl of the prepared standards, controls and samples for the Histamine ELISA**

6.4 Histamine ELISA

1.	Pipette **25 µl** of the **acylated standards, controls** and **samples** into the appropriate wells of the **Histamine Microtiter Strips**.
2.	Pipette **100 µl** of the **Histamine Antiserum** into all wells and cover plate with **Adhesive Foil.**
3.	Shake the **Histamine Microtiter Strips** briefly by hand and incubate for **20 - 25 h** at **2 - 8 °C**. ***Alternatively:*** *Incubate for 3 h at RT (20 - 25 °C) on a shaker (approx. 600 rpm).*
4.	Remove the foil, Discard or aspirate the content of the wells. Wash the plate **4 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **30 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm).
7.	Discard or aspirate the content of the wells. Wash the plate **4 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8. ⚠	Pipette **100 µl** of the **Substrate** into all wells and incubate for **20 - 30 min** at **RT** (20 - 25 °C) on a **shaker** (approx. 600 rpm). ***Avoid exposure to direct sunlight!***
9.	Add **100 µl** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

The standard curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

Controls
The concentrations of the **controls** can be read directly from the standard curve.

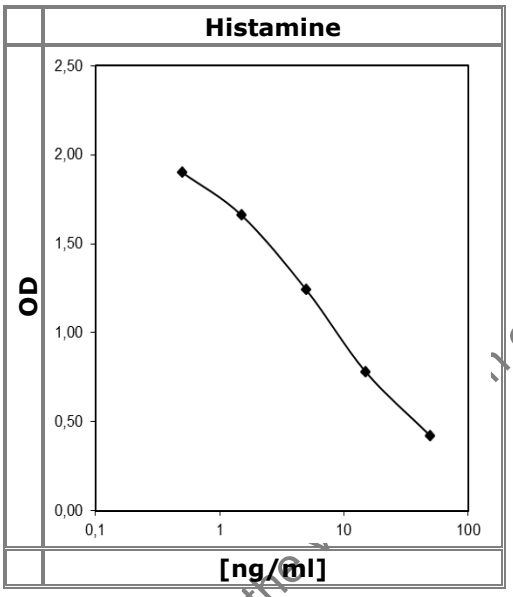
Samples
For this example (rat plasma) a sample pre-dilution of 1:21 was used. Therefore the concentrations read from the standard curve have to be **multiplied by 21.**
In general, if the samples have been pre-diluted, the concentrations read from the standard curve have to be multiplied by the dilution factor to get the final results. If no pre-dilution was necessary the final result could be read directly from the standard curve.

7.1 Quality control

The confidence limits of the kit controls are printed on the QC-Report.

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	Histamine
	0.2 ng/ml

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

Recovery and Linearity for different animal species (plasma samples):

Species	Recovery	Linearity
Mouse	Mean Recovery: 97% Range Recovery: 86 – 104 %	Mean Linearity: 115% Range Linearity: 94 - 134 %
Rat	Mean Recovery: 86% Range Recovery: 75 - 93 %	Mean Linearity: 115% Range Linearity: 88 - 131 %
Cat	Mean Recovery: 82% Range Recovery: 70 – 93 %	Mean Linearity: 115% Range Linearity: 94-134 %
Dog	Mean Recovery: 82% Range Recovery: 70 – 93 %	Mean Linearity: 115% Range Linearity: 94-134 %
Horse	Mean Recovery: 90% Range Recovery: 72 – 94 %	Mean Linearity: 115% Range Linearity: 94-134 %

⚠ ***For literature or any other information please contact your local supplier.***

⚠ ***The liability of the manufacturer shall be limited to the replacement of defective products. The manufacturer takes no liability for any damages or expenses arising directly or indirectly from the use of this product.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!