DRG® Histamine ELISA (EIA-4005)

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Revised 17 Nov. 2011 rm (Vers. 7.1) IVD

Please use only the valid version of the package insert provided with the kit.

Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Histamine in plasma and urine.
In combination with the supplementary kit (REF BA E-1100), the assay is performed for the histamine release in heparinized whole blood.

First, 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.

Advice on handling the test

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.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

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.

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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.
The appropriate safety data sheets of the individual products are available upon request. The safety data sheets correspond to the standard: ISO 11014-1.

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 testkit 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.

Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels.

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Contents of the kit

REAC-PLATE	*Reaction Plate*	***1 x 96 Wells***	***ready for use***
FOILS	***Adhesive Foil***	*1 x 4*	*ready for use*
WASH-CONC 50x	***Wash Buffer Concentrate***	*1 x 20 mL*	*concentrate, dilute content with dist. water to a final volume of 1000 mL*
SUBSTRATE	***Substrate***	*1 x 12 ml*	*ready for use, containing a solution of TMB*
STOP-SOLN	***Stop Solution***	*1 x 12 ml*	*ready for use, containing 0.25 M H_2SO_4*
STANDARD A	***Standard A***	*1 x 4 ml*	*ready for use*
STANDARD B	***Standard B***	*1 x 4 ml*	*ready for use*
STANDARD C	***Standard C***	*1 x 4 ml*	*ready for use*
STANDARD D	***Standard D***	*1 x 4 ml*	*ready for use*
STANDARD E	***Standard E***	*1 x 4 ml*	*ready for use*
STANDARD F	***Standard F***	*1 x 4 ml*	*ready for use*
HIS-AS	***Histamine Antiserum***	*1 x 12 ml*	*from goat, ready for use*
ACYL-BUFF	***Acylation Buffer***	*1 x 4 mL*	*ready for use*
ACYL-REAG	***Acylation Reagent***	*4 x 1.25 mL*	*lyophilised*
HIS	***Histamine Microtiter Strips***	*1 x 96 wells*	*12 strips, 8 wells each, break apart, pre-coated*
CONJUGATE	***Enzyme Conjugate***	*1 x 12 mL*	*ready for use, anti-goat IgG conjugated with peroxidase*

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CONTROL 1	***Control 1***	*1 x 4 mL*	*ready for use*
CONTROL 2	***Control 2***	*1 x 4 mL*	*ready for use*
ACYL-DILUENT	***Acylation Diluent***	*2 x 4 mL*	*ready for use*

Additional materials and equipment required but not provided in the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL /100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm and 620 or 650 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

Sample collection and storage

Plasma

Plasma (EDTA, Heparin) should be used. Haemolytic and especially lipemic samples should not be used with this assay.
Storage: 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.

Urine

Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, may be used.
Storage: 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.
Avoid exposure to direct sunlight.

Whole Blood

The histamine release is performed with heparinized whole blood. For further information please refer to the instructions for use of the Histamine Release ELISA (REF BA E-1100).

Test procedure

Allow all reagents and samples to reach room temperature prior to use.
The measurement in duplicates is recommended.

Preparation of reagents

Wash Buffer

Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 4 °C – 8 °C.

Acylation Diluent

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

Acylation Reagent

Reconstitute each vial with 1.25 mL Acylation Diluent.

The Acylation Reagent has to be prepared freshly prior to the assay (not longer than 1 hour in advance). If more than 1.25 mL is needed pool the contents of 2 or 3 vials and mix thoroughly.

Sample preparation and acylation

1. Pipette **25 µL** of **standards**, **25 µL** of **controls, 25 µL** of **plasma samples**, **10 µL** of **urine samples,** or **50 µL** of **supernatant** from the **release test*** into the respective wells of the **Reaction Plate.**
2. Add **25 µL** of **Acylation Buffer** to all wells.
3. Add **25 µL** of **Acylation Reagent** to all wells.
4. Incubate for **45 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
5. Add **200 µL** of **distilled water** to all wells.
6. Incubate for **15 min.** at **RT** (20-25°C) on a shaker (approx. 600 rpm).

Take **25 µL of the prepared standards, controls and samples** for the **Histamine ELISA**

* For the **release test** the **Histamine Release** supplementary kit (REF BA E-1100).has to be used.

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. Incubate for **3 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).

   Alternatively: *shake* the **Histamine Microtiter Strips** briefly by hand and incubate for **15 – 20 hours** at **2 – 8 °C**.

4. Remove the foil. Discard or aspirate the contents of the wells and **wash** each well **4 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 **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7. Remove the foil. Discard or aspirate the contents of the wells and **wash** each well **4 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 and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
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** with a reference wavelength between 620 nm and 650 nm.

Calculation of results

	Concentration of the standards					
Standard	***A***	***B***	***C***	***D***	***E***	***F***
Histamine (ng/ mL = µg/L)	*0*	*0.5*	*1.5*	*5*	*15*	*50*
Histamine (nmol/L)	*0*	*4.5*	*13.5*	*45*	*135*	*450*
Conversion:	*Histamine (ng/ mL) x 9 = Histamine (nmol/L)*					

The calibration 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).

Plasma samples and controls:
The concentrations of the **plasma samples** and the **controls** can be read directly from the standard curve.

Urine samples:
The read concentrations of **histamine in urine** have to be **multiplied by 2.5**
Calculate the 24 h excretion for each urine sample: **µg/24h = µg/L x L/24h**

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Quality control

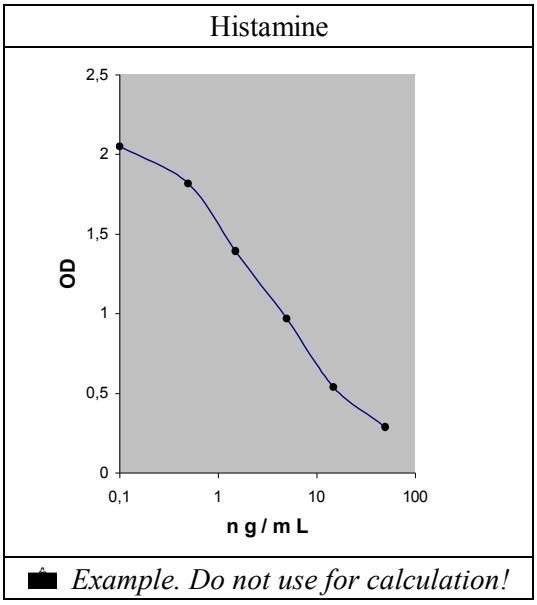
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit, or other commercially available, controls should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC-Report.

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. The extinction values also depend on the incubation times. 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

Typical calibration curve



Example. Do not use for calculation!

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Assay characteristics

Expected Reference Values	**Histamine**		
	Plasma	Urine	
		24 h	spontaneous
	< 1 ng/ mL	< 45 µg/d	< 45 µg/g creatinine

Analytical Sensitivity (Limit of Detection)	**Histamine**	
	Sensitivity Plasma	0.12 ng/ mL
	Sensitivity Urine	0.30 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

Precision							
Intra-Assay	Sample	Range (ng/ mL)	CV (%)	**Inter-Assay**	Sample	Range (ng/ mL)	CV (%)
Histamine	1	8.7 ± 0.6	7.4	Histamine	1	0.6 ± 0.1	12
Urine	2	30.1 ± 2.2	7.3	Control samples	2	4.6 ± 0.3	6.3
Histamine	1	2.03 ± 0.16	8				
Plasma	2	6.74 ± 0.37	5.6				

Linearity			Range	Serial dilution up to	Range (%)
	Histamine	Urine	4.33 - 70 ng/ mL	1:16	90 - 124
		Plasma	0.74 – 8.48 ng/ mL	1:16	85 -106

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Recovery			Mean (%)	Range (%)	% Recovery after spiking
	Histamine	Urine	112	108 - 123	
		Plasma	103	92 - 120	

Method comparison versus ELISA*	Histamine	Urine	ELISA*[1] = 0.9 ELISA*[2] – 3.1	r = 0.98; n = 29
		Plasma	ELISA*[1] = ELISA*[2] – 0.4	r = 0.99; n = 47

*[1] Immunotech ELISA *[2] This ELISA

For actual literature, information about clinical significance or any other information please contact your local supplier.

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