Product Manual

Histamine ELISA kit

Catalog #: ENZ-KIT140

96 Well Kit

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Product Manual

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Please read entire booklet before proceeding with the assay.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

The Enzo Histamine ELISA kit is a complete kit for the quantitative determination of histamine in human, mouse, rat and canine sample matrices. This competitive, ultra-sensitive ELISA does not require acylation of histamine and has a time-to-answer of 2 hours. Please read the complete kit insert before performing this assay.

Histamine is an endogenous short-acting biogenic amine synthesized from the amino acid histidine and is widely distributed throughout the body[1]. Histamine is an important mediator of immediate-type-allergic reactions[2]. Normally, there is a minimal amount of histamine circulating in the human body. An allergic reaction can occur when a substance enters the body (i.e. food, chemicals, insect bites, oils from plants). In response to the molecule, the body will then release larger quantities of histamine, which starts a cascade of events and begins an immune response. The body balances the histamine release by producing Epinephrine (adrenaline) which can help modulate the effects of histamine. When histamine is released, part of the cascade of events is inflammation. Antihistamines work by blocking the action of histamine and the resulting inflammation, amongst other symptoms, to provide relief.

Anaphylaxis occurs when there is a hypersensitive response. Reactions can range from mild to severe and, in extreme cases, can be fatal. Symptoms of hypersensitivity to histamine include hives, tingling feeling in the mouth, difficulty breathing, stomach cramping and diarrhea.

Elevations in plasma and tissue histamine levels have been reported during anaphylaxis and experimental systems investigating the allergic responses of the skin and airways[3]. Because of its potent role in the immune response, histamine in the body is present in a very transitory state and can only be measured within minutes of release. The half-life of histamine in a biological system is four minutes before conversion to n-methyl histamine[4]. N-methyl histamine is a major product of histamine metabolism, is present in urine and has a longer half-life than that of histamine[5]. This assay reacts equally to N-methyl histamine and histamine, making it an excellent choice for measuring histamine levels in urine.

Histamine is also in a class of neurotransmitters called “Small Molecule Neurotransmitter Substances”. This group includes molecules such as Serotonin, Epinephrine and Dopamine. Additionally, histamine plays a role in gastric acid secretion[6], assisting in the induction of acid production.

PRINCIPLE

1. Samples or standards are added to wells coated with a goat anti-rabbit IgG antibody. A polyclonal antibody to histamine and a solution of biotinylated histamine tracer are both added to the wells.
2. The plate is incubated. During this incubation, the antibody binds the histamine in the sample or tracer in a competitive manner.
3. The plate is washed, leaving only bound histamine on the plate. A solution of Horseradish Peroxidase conjugated Streptavidin (SA-HRP) is added to all wells and the plate is incubated.
4. The plate is washed to remove excess conjugate. TMB substrate is added to the wells and the plate is incubated.
5. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450 nm. The amount of signal is inversely proportional to the level of Histamine in the sample.

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

MATERIALS SUPPLIED

1. **Goat anti-Rabbit IgG Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-0060**

   A plate using break-apart strips coated with a goat anti-rabbit IgG antibody.

2. **Assay Buffer 13, 1x, 25 mL**
   Catalog No. 80-2305

   Tris buffered saline containing proteins and detergents.

3. **Histamine Tracer, Lyophilized**
   Catalog No. 80-2674

   Lyophilized biotinylated histamine tracer.

4. **Tracer Diluent, 6 mL**
   Catalog No. 80-2681

   Buffer for dilution of reconstituted histamine tracer.

5. **Histamine Antibody, Lyophilized**
   Catalog No. 80-2673

   Lyophilized polyclonal antibody specific to histamine.

6. **Antibody Diluent, 6 mL**
   Catalog No. 80-2680

   Buffer for dilution of reconstituted histamine antibody.

7. **Histamine Standard, 200 µL**
   Catalog No. 80-2672

   One vial containing 250 ng/mL histamine.

8. **Wash Buffer Concentrate, 100 mL**
   Catalog No. 80-1287

   Tris buffered saline containing detergents.

9. **SA-HRP Conjugate, 20 mL**
   Catalog No. 80-2675

   A solution of Streptavidin-conjugated Horseradish Peroxidase.

10. **TMB Substrate, 25 mL**
    Catalog No. 80-2101

    A solution of 3,3’5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide.

11. **Stop Solution 2, 10 mL**
    Catalog No. 80-0377

    A 1N solution of hydrochloric acid in water.

12. **Histamine Assay Layout Sheet, 1 each**
    Catalog No. 30-0328

13. **Plate Sealer, 3 each**

    Catalog No. 30-0012

STORAGE

All of the components of this kit are stable at 4°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing volumes between 50 µL and 200 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Microplate shaker
7. Adsorbent paper for blotting
8. Centrifugal concentrator (e.g. Speedvac™)
9. Methanol
10. Microplate reader capable of reading at 450 nm

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

SAMPLE HANDLING

This assay is suitable for measuring histamine in human, mouse and rat serum and EDTA plasma and human urine in addition to canine serum and tissue culture media. Prior to sample analysis in the assay, frozen samples should be slowly thawed at room temperature and vortexed to mix.

Neat (undiluted) methanol-extracted human serum and plasma samples, neat urine and tissue culture media in addition to mouse, rat and canine samples have been validated for use in this assay (please refer to the Spike and Recovery section on page 8 for detailed data). However, due to variation in samples, dilution *may* be required. Users must determine the optimal dilution(s) for their samples and experiments. The Methanol Precipitation Protocol for human serum and EDTA plasma samples is presented below. Extraction is necessary for serum and plasma samples having low amounts of endogenous histamine, where dilution greater than 1:10 would not produce a returned value.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

Methanol Precipitation Protocol for Human Serum and EDTA Plasma

1. Add 500 µL serum or EDTA plasma to an Eppendorf tube and add an equal volume of methanol to the same tube.
2. Vortex to mix and incubate on ice for 3 minutes.
3. Centrifuge samples at 5000 x g in a benchtop centrifuge for 5 minutes to clarify.
4. Transfer supernatant to a fresh Eppendorf tube and discard pellet. If the pellet is not firm, and the supernatant is still turbid, continue centrifugation for an additional 3 minutes before removing the supernatant.
5. Evaporate samples to dryness in a centrifugal concentrator for 2-3 hours at room temperature*. After drying, the extract will be a viscous, brown material of approximately 50-100 µL. If drying continues, it is possible to produce a pellet without any residual water, in which case the pellet is powdery and off-white in color.
6. Store the pellets at 4°C for short-term storage and at -20°C for long-term storage. When ready to assay the extracted sample, reconstitute the pellet in 500 µL of Assay Buffer 13 included in the kit. If required, the pellet can be reconstituted in a smaller volume to increase the amount of histamine reported. Reconstitution in 100 µL will give you a 5x extract and has been validated by Enzo Life Sciences.

   ***Note:** The application of heat may also be used during drying to achieve shorter drying times.

In the following experiments serum, plasma, urine and tissue culture media were utilized to determine the minimal recommended sample dilutions.

SAMPLE MATRIX PROPERTIES

Linearity

Methanol-extracted neat human serum and EDTA plasma samples and tissue culture media were spiked with histamine and serially diluted 1:2 in Assay Buffer 13. Human urine, canine serum and both mouse and rat serum and plasma samples with biologically relevant levels of histamine were serially diluted 1:2 in Assay Buffer 13. Additionally, non-extracted human serum and plasma were diluted 1:10, spiked with histamine and serially diluted 1:2 in Assay Buffer 13. All samples were run in the assay and compared to the standard curve. The results are shown in the table below.

Dilutional Linearity, %				
Dilution	**Human Serum (extracted)**	**Human Plasma (extracted)**	**Tissue Culture Media**	**Human Urine**
Neat	100	100	158	100
1:2	91	95	100	95
1:4	85	125	106	93
1:8	95	110	82	82

Dilutional Linearity, %					
Dilution	**Mouse Serum**	**Mouse Plasma**	**Rat Serum**	**Rat Plasma**	**Canine Serum**
Neat	---	---	---	---	---
1:2	100	100	---	100	100
1:4	76	90	---	89	89
1:8	75	90	100	87	118
1:16	76	81	87	92	---
1:32	80	86	91	97	---
1:64	---	92	103	114	---

Dilutional Linearity, %		
Dilution	**Human Serum (diluted and spiked)**	**Human Plasma (diluted and spiked)**
1:10	100	100
1:20	135	80
1:40	129	69
1:80	112	---

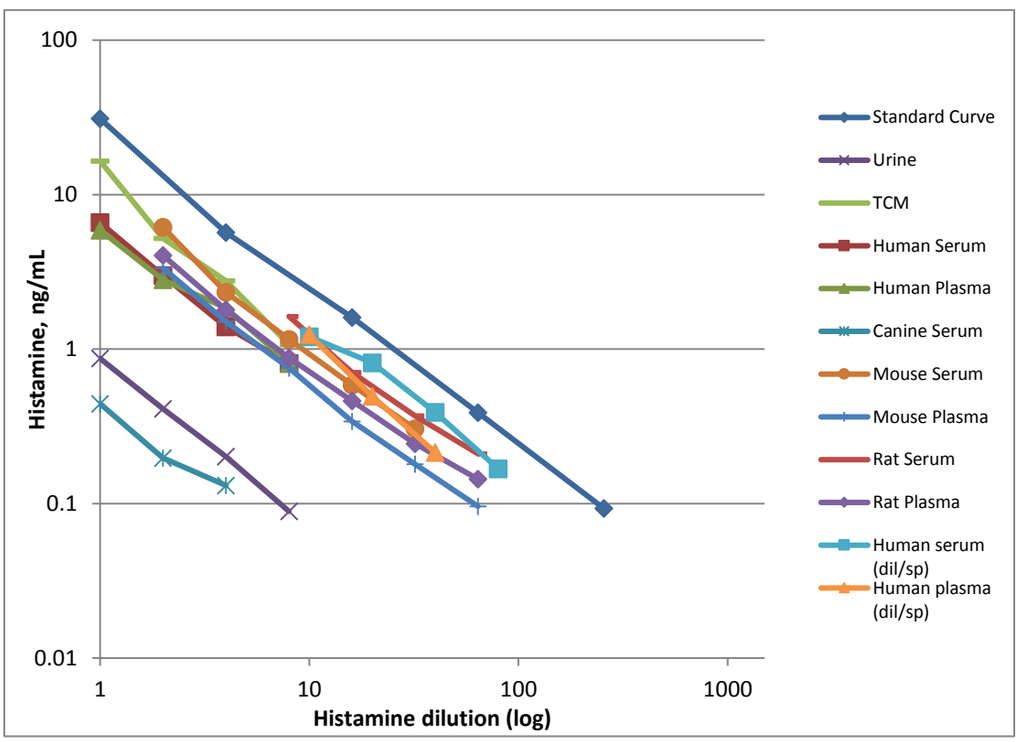
Spike and Recovery

Histamine was spiked at three concentrations into methanol-extracted neat human serum and EDTA plasma, canine serum and tissue culture media. N-methyl histamine, which is a primary urinary metabolite of histamine and is commonly measured in urine as a 1:1 indicator of histamine concentration and cross-reacts 100% in this assay, was spiked at three concentrations into neat urine. Matrix background was subtracted and the recovery was compared to the recovery of histamine spiked into Assay Buffer 13. The average percent recovery for each matrix at the minimum recommended dilution is indicated below.

Sample	Spike Concentration, ng/mL	% Recovery	Minimum Recommended Dilution
Serum (extracted)	5	131	Neat
	1	121	
	0.3	111	
EDTA Plasma (extracted)	5	148	Neat
	1	113	
	0.3	109	
Canine Serum	5	65	Neat
	1	108	
	0.3	103	
Tissue Culture Media	5	100	Neat
	1	102	
	0.3	44	
Urine	5	71.9	Neat
	1	104	
	0.3	115	

Parallelism

To assess parallelism, methanol-extracted human serum and EDTA plasma sample and tissue culture media were spiked with histamine and serially diluted in assay buffer. Additionally, human serum and plasma samples were diluted 1:10, spiked with histamine and serially diluted 1:2 in assay buffer. Neat human urine and canine serum samples were also serially diluted in assay buffer. Finally, mouse and rat serum and plasma samples at various dilutions were serially diluted in assay buffer. All of the samples were then run in the assay. The histamine concentration in each sample was determined from the standard curve. Concentrations were plotted as a function of sample dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples matrices of human, mouse, rat and canine origin.

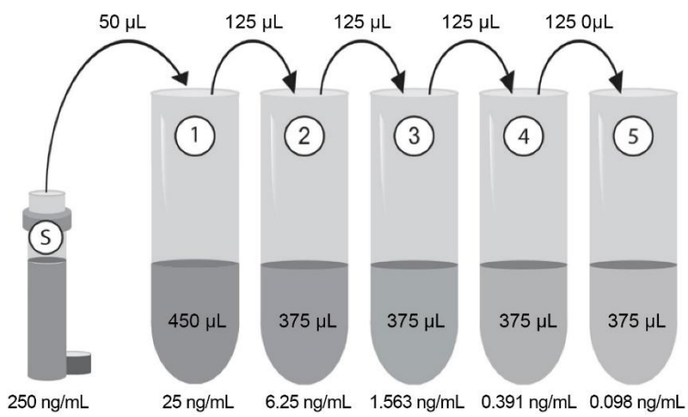


REAGENT PREPARATION

1. **Wash Buffer**
   Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Histamine Standard Curve**

   The histamine standard stock as well as diluted standards and samples should be kept on ice and used within 60 minutes of preparation for optimal performance. Allow the histamine standard to warm to room temperature.



   Label five 12x75 mm polypropylene tubes #1 through #5. Add 450 µL of Assay Buffer 13 into tube #1. Add 375 µL of Assay Buffer 13 into tube #2 through tube #5. Add 50 µL of 25 ng/mL histamine standard stock to tube #1 and vortex. Add 125 µL of tube #1 into tube #2 and vortex. Add 125 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 and #5.

   Diluted standards should not be stored for re-use. Make new standard preparations with each use.

3. **Histamine Tracer**

   Reconstitute the lyophilized histamine tracer in 600 µL Assay Buffer 13 to a 10x concentration. Dilute to 1x in Tracer Diluent within 30 minutes of running the assay. Store the unused reconstituted 10x concentrate at -20°C. It is stable for 3 freeze-thaw cycles.

4. **Histamine Antibody**

   Reconstitute the lyophilized histamine antibody in 600 µL Assay Buffer 13 to a 10x concentration. Dilute to 1x in Antibody Diluent within 30 minutes of running the assay.

Store the unused reconstituted 10x concentrate at -20°C. It is stable for 3 freeze-thaw cycles.

ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove unneeded wells and return them, with the desiccant, to the plate bag and seal. Store the unused wells at 4°C.

1. Add 100 µL of the assay buffer into the Bo (0 ng/mL standard) wells and 150 µL of the same assay buffer into the NSB wells.
2. Add 100 µL of standards #1 through #5 into the appropriate wells.
3. Add 100 µL of the samples into the appropriate wells.
4. Add 50 µL of the 1x histamine tracer to all wells except for the blank.
5. Add 50 µL of the 1x histamine antibody to all wells except for the NSB and blank.
6. Seal the plate and incubate at room temperature (RT) on a plate shaker for 1 hour at ~500 rpm*. **See note.**
7. Empty the contents of the wells and wash by adding full well volume, ~ 400 µL, of wash solution to every well. Repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Add 200 µL of SA-HRP conjugate into each well except the blank.
9. Seal the plate and incubate at RT on a plate shaker for 30 minutes at ~500rpm*.
10. Wash as above (Step 7).
11. Add 200 µL of TMB substrate solution into each well. Incubate for 30 minutes at RT on a plate shaker at ~500 rpm*.
12. Add 50 µL of the stop solution into each well.
13. After zeroing the plate reader against the blank, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well**

CALCULATION OF RESULTS

The concentration of histamine can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

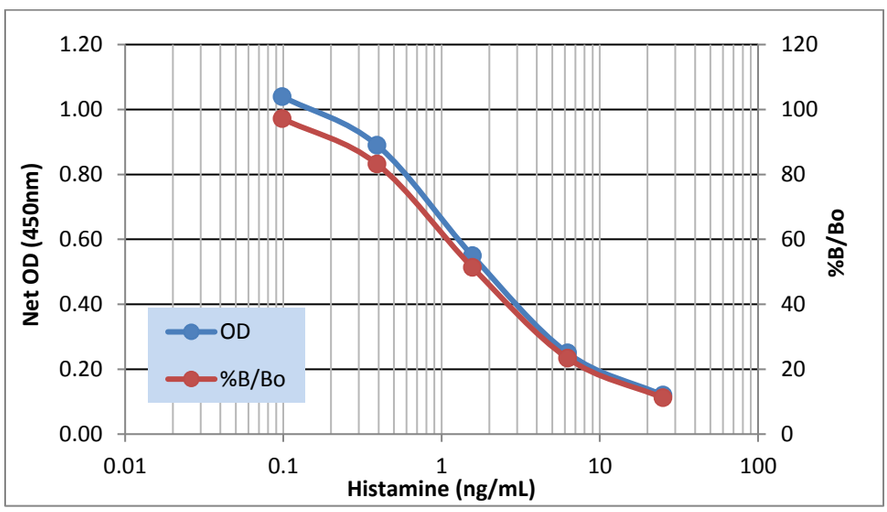
   Average Net OD = Average OD - Average NSB OD

2. Using data analysis software, plot the Average Net OD for each standard versus histamine concentration in each standard. We recommend that the data be handled by a software package utilizing a 4 parameter logistic (4PL) curve fitting program.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	Optical Density (450 nm)	Histamine (ng/mL)	%B/Bo
NSB	0.04	**n/a**	
Bo	1.07	**0**	
S1	0.12	**25**	**11.2**
S2	0.25	**6.25**	**23.4**
S3	0.55	**1.563**	**51.4**
S4	0.89	**0.391**	**83.2**
S5	1.04	**0.098**	**97.2**



PERFORMANCE CHARACTERISTICS

Specificity

The specificity of the assay was determined by diluting potential cross reactants in the kit assay buffer at a concentration of ten times the high standard and then measuring in the assay.

Analyte	Cross Reactivity
Histamine	100%
N-Methyl Histamine	100%
N-Acetyl Histamine	≤ 0.02%
L-Histidine	≤ 0.02%

Sensitivity

The sensitivity or limit of detection of the assay is 0.03 ng/mL. This was determined by interpolation at 2 standard deviations away from the net OD of a total of 20 zero standard replicates. Data was collected from 12 standard curves.

Intra-assay precision was determined by assaying 20 replicates of three controls containing histamine in a single assay.

Intra-assay precision	
ng/mL	**%CV**
4.62	7.1
0.89	4.9
0.23	8.4

Inter-assay precision was determined by measuring controls of varying histamine concentrations in multiple assays over several days.

Inter-assay precision	
ng/mL	**%CV**
4.73	8.9
0.94	4.8
0.26	7.9

REFERENCES

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NOTES

NOTES

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