ab213975 Histamine ELISA kit

For quantitative determination of Histamine in human, mouse, rat and canine sample matrices.
This product is for research use only and is not intended for diagnostic use.

Storage and Stability: Store ELISA kit at +2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Aliquot components in working volumes before storing at the recommended temperature.

Materials Supplied

Item	Quantity	Storage Condition before preparation	Storage Condition after preparation
Goat anti-Rabbit IgG coated microplate (12x 8 well strips)	96 wells	+2-8°C	+2-8°C
Assay Buffer	25 mL	+2-8°C	+2-8°C
Histamine Tracer (Lyophilized)	1 Vial	+2-8°C	-20°C
Tracer Diluent	6 mL	+2-8°C	+2-8°C
Histamine Antibody (Lyophilized)	1 Vial	+2-8°C	-20°C
Antibody Diluent	6 mL	+2-8°C	+2-8°C
Histamine Standard Stock (250 ng/mL)	200 µL	+2-8°C	+2-8°C
(20X) Wash Buffer	100 mL	+2-8°C	RT
SA-HRP Conjugate	20 mL	+2-8°C	+2-8°C
TMB Substrate	25 mL	+2-8°C	+2-8°C
Stop Solution	10 mL	+2-8°C	+2-8°C
Plate Sealer	3 units	+2-8°C	+2-8°C

Materials Required, Not Supplied

These materials are not included in the ELISA kit, but will be required to successfully perform this assay:

- Deionized or distilled water
- Precision pipettes for volumes between 5 µL and 1,000 µL
- Repeater pipette for dispensing volumes between 50 µL and 200 µL
- Disposable beakers for diluting buffer concentrates
- Graduated cylinders
- A microplate shaker
- Absorbent paper for blotting
- Centrifugal concentrator
- Methanol
- Microplate reader capable of reading at 450 nm

Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

Goat anti-Rabbit IgG coated microplate (12x 8 well strips): One plate (break-apart strips) of 96 wells with a goat anti-rabbit IgG antibody. Ready to use.
Assay Buffer: Ready to use.
Histamine Tracer (Lyophilized): Reconstitute the lyophilized histamine tracer in 600 µL Assay Buffer 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.
Tracer Diluent: Ready to use.
Histamine Antibody (Lyophilized): Reconstitute the lyophilized histamine antibody in 600 µL Assay Buffer 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.
Antibody Diluent: Ready to use.
Histamine Standard Stock (250 ng/mL): Ready to use.
(20X) 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.
SA-HRP Conjugate: Ready to use.
TMB Substrate: Ready to use.
Stop Solution: Ready to use.
Plate Sealer: Ready to use.

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

1. Label five 12x 75 mm polypropylene tubes #1 through #5.
2. Add 450 µL of Assay Buffer into tube #1.
3. Add 375 µL of Assay Buffer into tube #2 through tube #5.
4. Add 50 µL of 250 ng/mL histamine standard stock to tube #1 and vortex.
5. Add 125 µL of tube #1 into tube #2 and vortex.
6. Add 125 µL of tube #2 to tube #3 and vortex thoroughly.
7. Continue this for tubes #4 and #5.
8. Diluted standards should not be stored for re-use. Make new standard preparations with each use.

Standard#	Standard (µL)	Assay Buffer (µL)	Final volume (µL)	End Conc. (ng/mL)
1	50 Stock	450	375	25
2	125 µL Standard #1	375	375	6.25
3	125 µL Standard #2	375	375	1.563
4	125 µL Standard #3	375	375	0.39
5	125 µL Standard #4	375	500	0.098

Sample Preparation

- 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. 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 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.

Methanol Precipitation Protocol for 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.
   ΔNote: The sample can be used at both points.
6. Store the pellets at +2-8°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 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.
   ΔNote: 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.

Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at +2-8°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or "edge effects" have not been observed with this assay.
- Recommended plate layout:

Recommended plate layout

	1	2	3
A	B_S	Std 2	Sample 1
B	B_S	Std 2	Sample 1
C	NSB	Std 3	Sample 2
D	NSB	Std 3	Sample 2
E	B_0	Std 4	Etc
F	B_0	Std 4	Etc
G	Std 1	Std 5	
H	Std 1	Std 5	

Key:
B_S = Blank; contains substrate only.
NSB = Non-specific binding; contains assay buffer, histamine tracer, conjugate and substrate.
B_0 = 0 ng/mL standard; contains assay buffer, histamine tracer, conjugate, antibody and substrate.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate (see recommended plate layout).

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.
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 ~500 rpm.
10. Wash as above (Step 13.7).
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 450nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.
14. * The optimal speed for each shaker will vary. 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.

Calculations

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 Bound 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.

Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
Large CV Low sensitivity	Contaminated wash buffer	Prepare fresh wash buffer
	Improper storage of the kit	Store the all components as directed.

Technical Support

For all technical or commercial enquiries please go to:
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