Human Tryptase ELISA Kit

Sandwich Enzyme-Linked Immunosorbent Assay | Cat. No.: ARE6650

For quantitative detection of Human Tryptase concentrations in cell culture supernates, serum, and plasma.

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1. Introduction

Tryptases are serine proteases implicated in asthma and are highly expressed in human mast cells. They are derived from genes clustered on chromosome 16p13.3, including TPSAB1 and TPSB2. Elevated serum tryptase can occur in anaphylactic and anaphylactoid reactions, although a negative test does not exclude anaphylaxis.

2. Assay Principle

This kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human Tryptase in cell culture supernates, serum, and plasma. An antibody specific for Human Tryptase is coated on a 96-well plate. Standards and samples are added to the wells, and Human Tryptase present in the sample binds to the immobilized antibody. After washing, biotinylated anti-Human Tryptase antibody is added, followed by HRP-conjugated streptavidin. TMB substrate develops color in proportion to the amount of bound Tryptase, and absorbance is read at 450 nm.

3. Kit Components

Component	Volume
96-well Plate Coated With Anti-Human Tryptase Antibody	8 wells x 12 strips
Human Tryptase Standard	10 ng x 2
Biotin-Labeled Detection Antibody (100X)	120 µL
Streptavidin-HRP (100X)	120 µL
Standard/Sample Diluent	30 mL
Detection Antibody Diluent	12 mL
Streptavidin-HRP Diluent	12 mL
Wash Buffer (20X)	30 mL
TMB Substrate Solution	12 mL
Stop Solution	12 mL
Plate Adhesive Strips	3 strips
Instruction Manual	1 manual

4. Storage and Stability

All kit components are stable at 2-8°C. Standard should be stored at -20°C or -80°C after reconstitution; -80°C is recommended. Opened microplate wells or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

5. Materials Required But Not Provided

- Microplate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and pipette tips to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

6. Health and Safety Precautions

1. Reagents provided in this kit may be harmful if ingested, inhaled, or absorbed through the skin. Carefully review the MSDS for each reagent before conducting the experiment.
2. Stop Solution contains 2 N sulfuric acid (H2SO4) and is an extremely corrosive agent. Wear proper eye, hand, and face protection when handling this material. After the experiment, rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing of the plate.
3. Standard protein and Detection Antibody contain sodium azide as a preservative. Handle according to laboratory safety procedures.

7. Reagent Preparation

7.1 Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

1. Cell culture supernates: remove particulates by centrifugation. Assay immediately or aliquot and store samples at -20°C.
2. Serum: allow serum to clot in a serum separator tube for about 4 hours at room temperature. Centrifuge at approximately 1000 x g for 15 minutes. Analyze immediately or aliquot and store at -20°C.
3. Plasma: collect plasma using heparin or EDTA as anticoagulant. Centrifuge for 15 minutes at 1500 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C.
4. Cell lysates: collect cells and rinse with PBS. Homogenize and lyse cells thoroughly in lysate solution. Centrifuge at approximately 10000 x g for 5 minutes to remove debris. Assay aliquots immediately.
5. Bone tissue: extract demineralized bone samples in 4 M guanidine-HCl and protease inhibitors. Dissolve the final sample in 2 M guanidine-HCl.
6. Tissue homogenates: rinse tissue with 1X PBS to remove excess blood, homogenize in 20 mL of 1X PBS, and store overnight at <= -20°C. After two freeze-thaw cycles, centrifuge for 5 minutes at 5000 x g. Remove the supernate immediately for assay or aliquot and store at <= -20°C.
7. Urine: clear urinary samples by centrifugation and use directly without dilution. Store at -20°C.

Note: some lysis buffers, such as RIPA, cannot be used because some components may affect binding.

7.2 Standard Preparation

Reconstitute the lyophilized Human Tryptase Standard by adding 1 mL Standard/Sample Diluent to make the 10000 pg/mL standard stock solution. Allow the solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstitution. Two tubes of the standard are included in each kit. Use one tube for each experiment.

Standard	Add	Into
10000 pg/mL	-	-
5000 pg/mL	500 µL of the Standard (10000 pg/mL)	500 µL of the Standard/Sample Diluent
2500 pg/mL	500 µL of the Standard (5000 pg/mL)	500 µL of the Standard/Sample Diluent
1250 pg/mL	500 µL of the Standard (2500 pg/mL)	500 µL of the Standard/Sample Diluent
625 pg/mL	500 µL of the Standard (1250 pg/mL)	500 µL of the Standard/Sample Diluent
312 pg/mL	500 µL of the Standard (625 pg/mL)	500 µL of the Standard/Sample Diluent
156 pg/mL	500 µL of the Standard (312 pg/mL)	500 µL of the Standard/Sample Diluent
0 pg/mL	1 mL of the Standard/Sample Diluent	-

Note: standard solutions are best used within 2 hours. The 10000 pg/mL standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

7.3 Biotin-Labeled Detection Antibody Working Solution

Dilute the Biotin-Labeled Detection Antibody 1:100 with Detection Antibody Diluent and mix thoroughly. Prepare the solution no more than 2 hours before the experiment.

7.4 Streptavidin-HRP Working Solution

Dilute Streptavidin-HRP 1:100 with Streptavidin-HRP Diluent and mix thoroughly. Prepare the solution no more than 1 hour before the experiment.

7.5 Wash Buffer Working Solution

Pour the entire contents (30 mL) of Wash Buffer Concentrate into a clean 1,000 mL graduated cylinder. Bring the final volume to 600 mL with glass-distilled or deionized water (1:20).

8. Assay Procedure

The Streptavidin-HRP Working Solution and TMB Substrate Solution must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, mix completely and evenly. A standard detection curve should be prepared for each experiment. Users should determine sample dilution based on a crude estimation of protein amount in samples.

1. Prepare 7 wells for standards and 1 well for blank. Add 100 µL of each standard or sample into the appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
2. Remove the cover, discard solution, and wash the plate 3 times with Wash Buffer Working Solution. Let wash buffer remain in the wells for 1-2 minutes each time. Blot the plate onto absorbent material. Do not let wells completely dry at any time.
3. Add 100 µL Biotin-Labeled Detection Antibody Working Solution into each well. Incubate for 1 hour at room temperature with gentle shaking.
4. Wash the plate 3 times with Wash Buffer Working Solution.
5. Add 100 µL Streptavidin-HRP Working Solution into each well. Incubate for 45 minutes at room temperature with gentle shaking.
6. Wash the plate 5 times with Wash Buffer Working Solution.
7. Add 100 µL TMB Substrate Solution into each well and incubate in the dark for 10-20 minutes.
8. Add 100 µL Stop Solution into each well. The color changes to yellow immediately.
9. Read OD absorbance at 450 nm within 30 minutes after adding Stop Solution.

9. Assay Procedure Summary

Step	Operation
1	Prepare all reagents, samples and standards.
2	Add 100 µL Standard or Sample.
3	Wash plate 3 times with Wash Buffer Working Solution.
4	Add 100 µL Biotin-Labeled Detection Antibody Working Solution.
5	Wash plate 3 times with Wash Buffer Working Solution.
6	Add 100 µL Streptavidin-HRP Working Solution.
7	Wash plate 5 times with Wash Buffer Working Solution.
8	Add 100 µL TMB Substrate Solution.
9	Add 100 µL Stop Solution.
10	Read the plate at 450 nm.

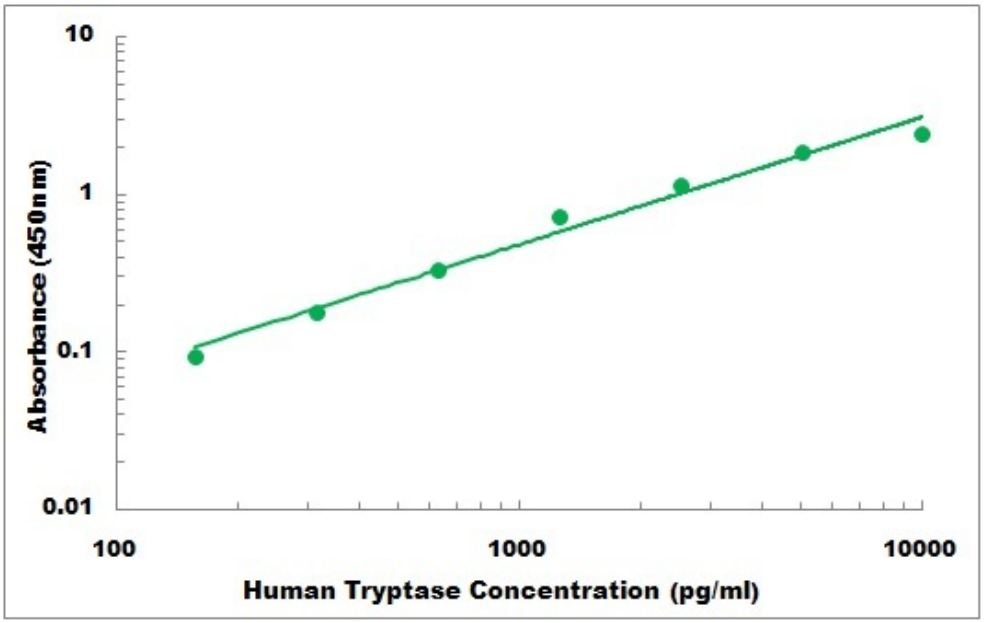
10. Calculation of Results

For calculation, relative OD450 = OD450 of each well - OD450 of the zero well. Plot the standard curve as the relative OD450 of each standard solution (Y) versus the respective concentration of the standard solution (X). The concentration of samples can be interpolated from the standard curve.

Note: if measured samples were diluted, multiply the interpolated concentration by the dilution factor to obtain the concentration before dilution.

11. Typical Data

The standard curve is for demonstration only. A standard curve must be run with each assay.



12. Sensitivity

The minimum detectable dose of Human Tryptase is typically less than 15 pg/mL.

13. Specificity

The Human Tryptase ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human Tryptase proteins within the range of 156 pg/mL - 10000 pg/mL.

14. Cross Reactivity

No detectable cross-reactivity with other relevant proteins.

15. Troubleshooting Guide

If the ELISA result is unsatisfactory, store the OD data, used strips, and remaining reagents. Refer to the following table to check possible reasons.

Problem	Possible Cause	Solution
High signal and background in all wells	Insufficient washing; too much Streptavidin-HRP; incubation time too long; development time too long.	Increase number of washes and soaking time; check dilution or titration; reduce incubation or development time.
No signal	Reagent added in incorrect order or incorrectly prepared; standard has gone bad; assay conducted from an incorrect starting point.	Review protocol; check stored standard condition; allow reagents to come to 20-30°C before assay.
Too much signal; whole plate turned uniformly blue	Insufficient washing with unbound Streptavidin-HRP remaining; too much Streptavidin-HRP; reused plate sealer or reservoir.	Increase number of washes carefully; check dilution; use fresh plate sealer and reagent reservoir for each step.
Standard curve achieved but poor discrimination between points	Plate not developed long enough; improper standard-curve dilution calculation.	Increase substrate-solution incubation time; check dilution and prepare a new standard curve.
No signal when expected, but standard curve looks fine	Sample matrix is masking detection.	Use a more diluted sample.
Samples read too high, but standard curve is fine	Samples contain protein levels above assay range.	Dilute samples and run again.
Edge effect	Uneven temperature around work surface.	Avoid incubating plate where environmental conditions vary; use plate sealer.

16. Notes

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