ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Autotaxin ELISA Kit

(Catalog Number: 31770)

For the quantitative determination of mouse autotaxin concentrations in serum or plasma samples

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INTRODUCTION

Autotaxin, also known as ENPP-2, is a secreted glycoprotein which belongs to the ectonucleotide pyrophosphatase/phosphodiesterase (NPP) family[1,2]. Generally, NPPs can hydrolyze phosphates from nucleotides. Autotaxin exhibits the unique lysophospholipase D activity[3]. The mature protein includes two somatomedin-B-like (SMB) cysteine knot domains, a catalytic domain, and an inactive C-terminal nuclease-like domain with an EF-hand-like motif that is important in cell motility, and a region involved in autotaxin secretion[1,4,5]. There are three isoforms identified in mouse and human[6,7]. Most circulating autotaxin is the β form which contains 863 amino acids. Autotaxin contributes to the predominant extracellular source of the phospholipid LPA (lysophosphatidic acid) from LPC (lysophosphatidylcholine)[8-11]. Autotaxin can also produce minor amounts of sphingosine 1-phosphate and cyclic phosphatidic acid which can antagonize many of the tumorigenic properties of LPA[9,12]. Autotaxin stimulates tumor cell motility and enhances invasion and metastasis. It is upregulated in melanoma, glioblastoma, breast and lung carcinoma, follicular lymphoma and other cancers[2,8,11]. Autotaxin production by adipocytes enhances pre-adipocyte proliferation and may be elevated in obesity[11,13]. Autotaxin is present in blood, urine, saliva, seminal and cerebrospinal fluids[2,3]. In addition, plasma autotaxin is cleared by the liver, which is elevated in liver disease[3,14]. Normal serum or plasma autotaxin concentration is reported to be slightly higher in females than in males, and highest in pregnant females[2,14].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with affinity purified polyclonal antibody against human autotaxin. Standards and samples are pipetted into the wells and any human autotaxin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody specific for human autotaxin is added to the wells. After wash step to remove any unbound reagents, streptavidin-HRP conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution 3,3′,5,5′-Tetramethylbenzidine (TMB) is added, and color develops in proportion to the amount of human autotaxin bound initially. Color reaction is stopped by 2M H_2SO_4 and the optical density of the wells are determined using a microtiter plate reader at 450nm. Since the increases in absorbance are directly proportional to the amount of captured human autotaxin, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human Autotaxin ELISA kit is designed for the detection and quantitative measurement of human autotaxin in serum or plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a rabbit polyclonal antibody against human autotaxin, sealed

2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 30 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against human autotaxin, 0.12 mL
5. Human autotaxin standard, 50 ng of recombinant human autotaxin, lyophilized.
6. 200×STP-HRP solution, 0.06 mL
7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the human autotaxin microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (30 mL) with 120 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 mL) with 450 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

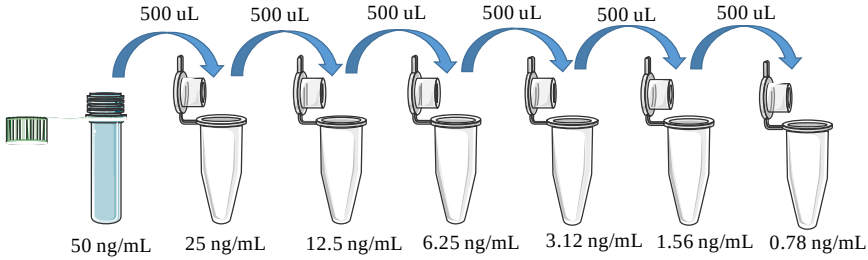
D. 1×STP-HRP solution

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution

is required per well. Prepare only as much 1×STPHRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS

Human Autotaxin Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 50 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 25, 12.5, 6.25, 3.12, 1.56, 0.78 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and stored at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **100-fold** dilution in the 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be run in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 2 hours.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**

8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

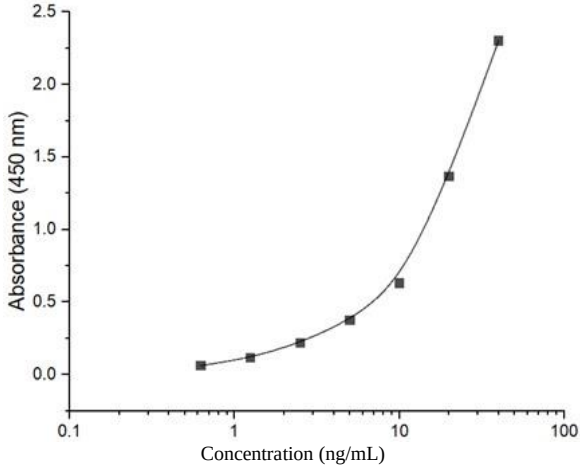
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human ENPP2 (ATX) concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human ENPP2 (ATX) concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

Human autotaxin (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.061	0
0.78	0.106	0.045
1.56	0.141	0.08
3.12	0.222	0.161
6.25	0.368	0.307
12.5	0.656	0.595
25	1.191	1.13
50	2.355	2.294

Human autotaxin standard curve (4-parameters)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest human autotaxin level that can be measured by this assay is 0.78 ng/mL.

B. Specificity

No cross reactivity or interference was observed when tested with recombinant mouse autotaxin protein.

C. Precision

Intra-assay Precision (Precision within an assay) C.V.: 5%.
Inter-assay Precision (Precision between assays) C.V.: 6.1%.

D. Spiking

Serum samples were assayed by adding 90 µL of sample and 10 µL of spike stock solution calculated to yield the intended 0, 5, 10 ng/mL spike concentration. The recovery of human autotaxin falls between 90-110%.

E. Linearity

To assess the linearity of the assay, samples spiked with high concentrations of human autotaxin were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

Dilution	Measured ng/mL	Expected ng/mL	Recovery %
1:40	1284	1284	100
	856.8	856.8	100

	1151	1151	100
1:80	1337.1	1284	104.1
	881	856.8	102.8
	1216	1151	105.6

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SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 2 hours.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1xDetection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1xSTP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation