Mouse Autotaxin immunoassay kit

Catalogue number: 32770

For the quantitative determination of autotaxin
in serum, plasma and fluid

This package insert must be read in its entirety before using this product

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TABLE OF CONTENTS

Contents	Page
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	1
REAGENTS SUPPLIED	2
OTHER MATERIALS REQUIRED, BUT NOT PROVIDED	2
STORAGE	2
PREPARATION OF REAGENTS	3

PREPARATION OF STANDARDS AND SAMPLES	4
SAMPLE HANDLING	4
ASSAY PROCEDURE	5
CALCULATION	5
TYPICAL STANDARD CURVE	6
ASSAY CHARACTERISTICS	7
REFERENCE	7
SUMMARY OF ASSAY PROCEDURE	9

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 acid. 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's 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 ELISA.The micro-plate is pre-coated with a rabbit polyclonal antibody against mouse autotaxin. Standards and samples are pipetted into the wells and any mouse autotaxin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin

labelled polyclonal antibody specific for mouse 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 is added and colour develops in proportion to the amount of mouse autotaxin bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured mouse autotaxin, the unknown sample concentration can be interpolated from a reference curve included in each assay

INTENDED USE

This mouse autotaxin ELISA kit is designed for quantification of mouse autotaxin in serum, plasma and cell culture supernate samples.

REAGENTS SUPPLIED

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

1. Micro-titre Strips (96 wells)-Coated with a rabbit polyclonal antibody against mouse 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 mouse autotaxin, 0.12 ml.
5. Mouse autotaxin standard-40 ng of recombinant mouse autotaxin in a buffered protein base, lyophilised.
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 micro-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×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.

B. 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.

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 STANDRADS AND SAMPLES

Mouse autotaxin standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 40 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1x Assay buffer	Final concentration
40 ng/ml stock	-	40 ng/ml
250 ul of 40 ng/ml	250 ul	20 ng/ml
250 ul of 20 ng/ml	250 ul	10 ng/ml
250 ul of 10 ng/ml	250 ul	5 ng/ml
250 ul of 5 ng/ml	250 ul	2.5 ng/ml
250 ul of 2.5 ng/ml	250 ul	1.25 ng/ml
250 ul of 1.25 ng/ml	250 ul	0.625 ng/ml

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 HANDLING

Different samples may require dilution. Serum samples requires a suggested 100-fold dilution. The certain dilution factor needs to be considered according to your requirement.

Assay procedure

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

1. Add 100 µl of standard or sample per well, incubate at room temperature for 1 hour.
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 mouse autotaxin 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 mouse autotaxin 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.

Mouse autotaxin (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.078	0
0.625	0.141	0.063
1.25	0.194	0.116

2.5	0.297	0.219
5	0.452	0.374
10	0.708	0.63
20	1.446	1.368
40	2.381	2.303

Mouse autotaxin standard curve (4-parameters)

Concentration (ng/ml)

ASSAY CHARACTERISTICS

A. Sensitivity

The lowest mouse autotaxin level that can be measured by this assay is 0.312 ng/ml.

B. Precision

Intra-assay Precision (Precision within an assay) C.V <10%.
Inter-assay Precision (Precision between assays) C.V <10%.

C. 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 spiked to different levels falls in 80-120%.

D. Validations

8 weeks-old C57BL mice fed with 8 week standard chow or high-fat diet.
The serum levels of autotaxin were determined.

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summary OF Assay procedure

Add 100 µl of standard or sample to each well.

Incubate at room temperature for 1 hour.

Aspirate and wash each well three times.

Add 100 µl of 1x Detection antibody solution to each well.

Incubate at room temperature for 1 hour.

Aspirate and wash each well three times.

Add 100 µl of 1x STP-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