Echelon Biosciences Inc.

Autotaxin (ATX) Sandwich ELISA

K-5600 (96 tests)

Support: echelon@echelon-inc.com

Description: 96-well ELISA Assay for Detection and Quantification of ATX in cells or biological fluids

Materials Provided

Catalog #	Description	Amount
K-5601	ATX Detection Plate	1 plate
K-5602	ATX Standard (0.1 µg Lyophilized)	1 vial
K-5603	Anti-ATX Antibody	1 bottle
K-SEC7	Secondary Detector (0.35 mL)	1 vial
K-PBSTB	5X Diluent (10 mL)	1 bottle
K-PBST3	10X PBS-T Buffer (30 mL)	1 bottle
K-TMB1	TMB Solution (12 mL)	1 bottle
K-STOPt	1N H_2SO_4 Stop Solution (10 mL)	1 bottle
---	Microtiter plate seal	3 seals

Quick Protocol

Prepare reagents, samples, and standards.

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Add 100 µL of standards and samples to the Detection Plate (K-5601) and incubate for one hour at RT.

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Wash the plate 3 times with 200 µL/well 1x PBST.

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Apply 100 µL/well diluted anti-ATX antibody (K-5603) to the Detection Plate and incubate for 1 hour at RT.

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Wash the plate 3 times with 200 µL/well 1x PBST.

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Apply 100 µL/well diluted Secondary Detector (K-SEC7) to the Detection Plate and incubate for 1 hour at RT.

↓

Wash the plate 3 times with 200 µL/well 1x PBST.

↓

Add 100 µL TMB solution to each well and incubate for 30 minutes at room temperature in the dark.

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Add 50 µL/well Stop Solution and read absorbance at 450 nm.

Additional Materials Provided by User

- Microtiter plate reader capable of reading absorbance at 450 nm.
- Pipettes (20 µL, 200 µL, and 1,000 µL)
- Micro centrifuge tubes for samples and standards dilution.
- Reagent grade water

Storage

Upon receipt store kit Part 1 at -20°C and Part 2 (K-TMB1) at 4°C.

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Background

Autotaxin (ATX), also known as ENPP2, lysophospholipase D (lysoPLD), phosphodiesterase 1α and plasma cell glycoprotein-1, is a secreted glycoprotein that is widely expressed with high levels in the serum. Via its lysoPLD activity, autotaxin hydrolyzes lysophosphatidylcholine (LPC) to generate the phospholipid growth factor lysophosphatidic acid (LPA). The enzyme's same activity hydrolyzes sphingosylphosphorylcholine (SPC) to form sphinosine-1-phosphate (S1P). Autotaxin was first isolated as the autocrine motility factor secreted from melanoma cells[1] and was subsequently shown to be identical to serum lysoPLD[1,2,3]. Since then, the cancer-related activities of autotaxin, at least in cultured cells, have been attributed to the enzyme's lysoPLD activity[4,5]. In addition to cancer, autotaxin has been implicated in a number of diseases including obesity, arthritis, multiple sclerosis, Alzheimer's disease and neuropathic pain[6].

Assay Design

The ATX Sandwich ELISA is a quantitative immunoassay designed for in vitro measurement of ATX levels in cell culture supernatant, or human biological fluids. The concentration of ATX in the sample is determined using a standard curve of known amounts of ATX. The assay was validated with sera, plasma, urine and ovarian asecites. The ATX Sandwich ELISA provides a robust and simple method for researchers to measure ATX in biological samples.

Assay Notes

1. The ATX Sandwich ELISA is designed for one-time use only. Collect enough samples to ensure the kit is fully utilized. If the assay kit is needed to be run twice, the reconstituted ATX Standard (K-5602) and Anti-ATX Antibody (K-5603) can be stored at -20 °C for a week but a reduction in assay signal may be observed. Always run a full ATX standard curve to ensure accurate results.
2. Suggested dilution for the tested types of sample are listed below in Table 1. Customer should determine the sample dilution factor if another type of sample is tested. If sample results with concentration higher than the highest ATX standard (100 ng/mL), further dilute the samples with the 1X Diluent and repeat the assay.

Table 1

Species	Type	Dilution
Human	Serum	1:50
Human	Plasma	1:50
Human	Ascites	1:50
Human	Urine	< 1:5
Mouse	Plasma	1:10
Mouse	Aveolar Lavage	Neat
Mouse	Homogenates	1:2 (2mg/mL protein)
Cell Culture	Supernatant	1:5

3. Types of sample tested in this assay are listed below in Table 2. No medical histories were available for the tested donors. The concentrations detected with the ATX Sandwich ELISA are comparable to the reported values from literature[7]. Intra and Inter assay coefficient of variations (CV) were calculated from duplicates in two separate assays.
4. Assay interference was tested on recombinant human and mouse ENPP7, and recombinant mouse ATX at 500 ng/mL concentration spiked with ATX. No cross reactivity was observed on the recombinant human and mouse ENPP7. The ATX Sandwich ELISA can detect recombinant mouse ATX and ATX in normal mouse and rat samples. Recombinant mouse ATX standard performed similar to the human ATX standard provided in the kit. However, no further evaluation was done on mouse samples. Until all factors have been tested the possibility of interference cannot be excluded.
5. The ATX Sandwich ELISA minimal detectable dose (MDD) of ATX is 3.21 ng/mL. The MDD was determined by adding three standard deviations to the mean optical density of two zero standard replicates and calculating the mean corresponding concentration from 5 individual assays.
6. The data is best analyzed by fitting the ATX Standards using "Linear Regression" curve fit on a log-log plot. Alternatively, ATX Standards can be fitted using "Sigmoidal Doses Response" curve fit on a log-log or semi-log plot and Second Order Polynomial curve fit using regular plot. No significant different between the type of curve fits use on calculating samples corresponding ATX concentration. However, same method of analysis should be used between experiments for more accurate comparison.

Table 2

Sample Types	Mean (ng/mL)	Range (ng/mL)	Intra Assay CV	Inter Assay CV
Healthy Human Serum	870	589 - 1135	3%	9%
Healthy Human Citrate Plasma	684	465 - 911	4%	3%
Healthy Human EDTA Plasma	822	544 - 1106	4%	8%
Healthy Human Heparin Plasma	926	622 - 1266	15%	8%
Serum/Plasma (possible cancer patients)	1614	1012 - 2792	4%	5%
Ovarian Cancer Patients Ascites	1700	410 - 2919	6%	6%
Healthy Human Urine	7.8	ND* - 11.2	11%	9%
Cell Culture Supernatant	22.9	9.6 – 67.9	17%	N/A
Mouse Plasma	208	140 – 269	6%	N/A
Mouse Bronchoalveolar Lavage	6.3	2.1 – 10.8	13%	N/A
Mouse Tumor Homogenates	52.1	3.0 – 160.0	6%	N/A

Assay Protocol

Please read this entire section and Assay Notes before beginning. The assay procedure was designed for samples/controls to be run in duplicate

1. Place ATX Standard (K-5602), Anti-ATX Antibody (K-5603) and Secondary Detector (K-SEC7) on ice until use. Bring remaining reagents to room temperature. Centrifuge vials before opening.
2. Prepare 1X PBST wash buffer by diluting 30 mL of the 10X PBST (K-PBST3) with 270 mL nano pure water. Keep at RT.
3. Prepare 1X Diluent by diluting 10 mL of the 5X Diluent (K-PBSTB) with 40 mL nano pure water. Keep at RT.
4. Dilute samples in 1X Diluent. Pipette up and down to mix. Suggested dilution for human serum/plasma is 1:50 (5 µL of human serum/plasma + 245 µL 1X Diluent). See assay notes #2 and #3 for more information on sample dilutions.
5. Prepare 8-point ATX Standard Curve in tubes as follows:
   a. Add 1 mL of 1X Diluent into the ATX Standard (K-5602) for final concentration at 100 ng/mL. Invert tube up and down to mix. DO NOT VORTEX. Incubate on ice for minimal of 5 minutes to fully reconstitute the ATX Standard. Keep on ice until the next step. See assay note #1 for standard stability once diluted.
   b. Then, prepare the 8-point ATX Standard as shown in Table 3.
6. Remove the ATX detection plate (K-5601) from plastic bag. Add 100 µL/well of the diluted samples and prepared standards to the ATX Detection Plate (K-5601). Suggested plate layout is shown in Table 4. Tap plate to mix. Cover plate with new plate seal. Incubate at room temperature for 1 hour.

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Table 3, Standard Dilutions

ATX [C]	Previous Dilution Needed	1X Diluent Needed
100 ng/mL	0.25 mL of 100 ng/mL	None
50 ng/mL	0.25 mL of 100 ng/mL	0.25 mL
25 ng/mL	0.25 mL of 50 ng/mL	0.25 mL
12.5 ng/mL	0.25 mL of 25 ng/mL	0.25 mL
6.25 ng/mL	0.25 mL of 12.5 ng/mL	0.25 mL
3.125 ng/mL	0.25 mL of 6.25 ng/mL	0.25 mL
1.5625 ng/mL	0.25 mL of 3.125 ng/mL	0.25 mL
0 ng/mL	None	0.25 mL

Table 4, Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	ATX Std. 100 ng/mL	ATX Std. 100 ng/mL	Sample #1	Sample #1	Sample #9	Sample #9	Sample #17	Sample #17	Sample #25	Sample #25	Sample #33	Sample #33
B	ATX Std. 50 ng/mL	ATX Std. 50 ng/mL	Sample #2	Sample #2	Sample #10	Sample #10	Sample #18	Sample #18	Sample #26	Sample #26	Sample #34	Sample #34
C	ATX Std. 25 ng/mL	ATX Std. 25 ng/mL	Sample #3	Sample #3	Sample #11	Sample #11	Sample #19	Sample #19	Sample #27	Sample #27	Sample #35	Sample #35
D	ATX Std. 12.5 ng/mL	ATX Std. 12.5 ng/mL	Sample #4	Sample #4	Sample #12	Sample #12	Sample #20	Sample #20	Sample #28	Sample #28	Sample #36	Sample #36
E	ATX Std. 6.25 ng/mL	ATX Std. 6.25 ng/mL	Sample #5	Sample #5	Sample #13	Sample #13	Sample #21	Sample #21	Sample #29	Sample #29	Sample #37	Sample #37
F	ATX Std. 3.125 ng/mL	ATX Std. 3.125 ng/mL	Sample #6	Sample #6	Sample #14	Sample #14	Sample #22	Sample #22	Sample #30	Sample #30	Sample #38	Sample #38
G	ATX Std. 1.563 ng/mL	ATX Std. 1.563 ng/mL	Sample #7	Sample #7	Sample #15	Sample #15	Sample #23	Sample #23	Sample #31	Sample #31	Sample #39	Sample #39
H	ATX Std. 0 ng/mL	ATX Std. 0 ng/mL	Sample #8	Sample #8	Sample #16	Sample #16	Sample #24	Sample #24	Sample #32	Sample #32	Sample #40	Sample #40

7. Discard solution from plate, wash plate three times with 200 µL 1X PBST wash buffer per well.
8. Prepare the Anti-ATX Antibody by adding 12 mL 1X Diluent into the Anti-ATX Antibody (K-5603) bottle. Invert up and down to mix. Add 100 µL/well to the ATX Detection Plate (K-5601). Tap plate to mix. Cover with new plate seal and incubate at room temperature for 1 hour. See assay note #1 for information on antibody stability once diluted.
9. Discard solution from plate, wash plate three times with 200 µL 1X PBST wash buffer per well.
10. Prepare the Secondary Detector by mixing 0.3 mL of the Secondary Detector (K-SEC7) with 12 mL 1X Diluent. Mix well. Add 100 µL/well to the ATX Detection Plate (K-5601). Tap plate to mix. Cover with new plate seal and incubate at room temperature for 1 hour.
11. Discard solution from plate, wash plate three times with 200 µL 1X PBST wash buffer per well.
12. Add 100 µL TMB solution per well (K-TMB1). Let blue color develop for approximately 30 minutes. Protect from light.
13. Add 50 µL 1 N H_2SO_4 solution (K-STOPt) to each well to stop the reaction. Tap plate to mix.
14. Read absorbance at 450 nm.
15. Generate a best fit curve for standards in order to interpolate relative sample values. See Figure 1 and assay note #6 for more information.

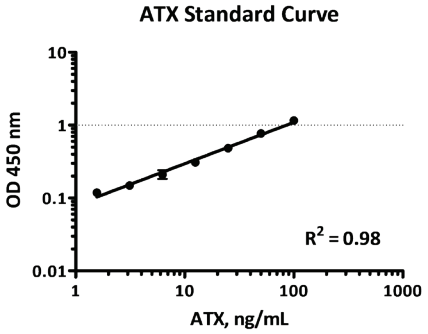

Figure 1.
ATX Standard Curve was generated using linear regression in GraphPad by plotting both x-axis and y-axis in log-scale.

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References (Assay Background)

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7. Nakamura, K. et al. Clinica Chimica Acta 388 (1-2), 51–58 (2008).

References (Product Publications)

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2. Lee, B.-H., et al. Biol and Pharm Bull, 40(7): p. 1063-1070 (2017)
3. Meng, G., et al., Faseb J, (2017)
4. Nsaibia, M.J., et al. J Int Med (2016)
5. Black, K.E., et al. Faseb J, (2016)
6. Bouchareb, R., et al. Aortic Valve. Circulation (2015)
7. Benesch, M.G.K., et al. J Lipid Res (2015)

Related Products

Catalog #	Products
Assays and Services	
T-4100	Autotaxin Activity Assay Service
K-4100	Autotaxin Activity Assay
K-4200 (HTS)	Autotaxin InhibitorScreening Kit
ATX Inhibitors	
B-0701	HA130
B-0702	PF-8380
L-3282	S32826
L-3223	GWJ-23
L-7118	Thio-ccPA
L-7218	Oleoyl-3-carbacyclic phosphatidic acid
L-7416	BrP-LPA
Enzymes and Substrates	
E-4000	Autotaxin enzyme, active
L-2000	FS-3 (fluorogenic lysoPLD substrate)
L-2010	ATX-Red AR-2

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