

# Echelon Biosciences Inc.

## Autotaxin (ATX) Sandwich ELISA

K-5600 (96 tests)

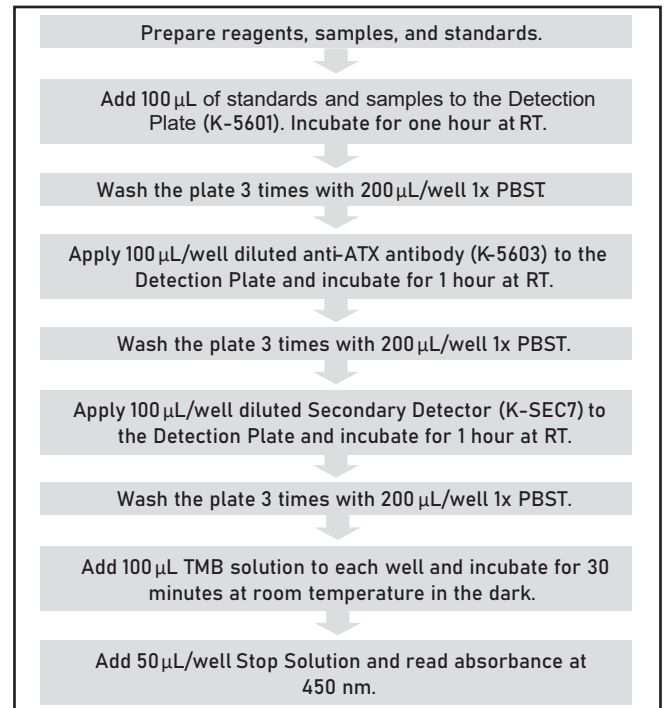
Support: echelon@echelon-inc.com

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 (0.1µg 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 <sub>2</sub> SO <sub>4</sub> Stop Solution (8 mL)	1 bottle
---	Microtiter plate seal	3 seals

### Quick Protocol



### 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 at -20°C.

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## Background

Autotaxin (ATX), also known as ENPP2, lysophospholipase D (lyso-PLD), phosphodiesterase 1 $\alpha$  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 sphingosine-1-phosphate (S1P). Autotaxin was first isolated as the autocrine motility factor secreted from melanoma cells<sup>1</sup> and was subsequently shown to be identical to serum lysoPLD<sup>1,2,3</sup>. Since then, the cancer-related activities of autotaxin, at least in cultured cells, have been attributed to the enzyme's lysoPLD activity<sup>4,5</sup>. In addition to cancer, autotaxin has been implicated in a number of diseases including obesity, arthritis, multiple sclerosis, and Alzheimer's disease<sup>6</sup>.

## 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 ascites. The ATX Sandwich ELISA provides a robust and simple method for researchers to measure ATX in biological samples.

## Assay Notes

1. If the assay is to be run twice, the reconstituted ATX Standard (K-5602) and Anti-ATX Antibody (K-5603) can be stored at 4°C for a month. The TMB (K-TMB1) should be stored at 4°C. The 1X PBST3 buffer and stop solution (K-STOPT) can remain at room temperature. The remaining reagents should be stored at -20°C until next use. A reduction in assay signal may be observed. Always run a full ATX standard curve to ensure accurate results.
2. Suggested dilution for tested samples are listed in Table 1. Customer should determine the sample dilution for sample types not listed. 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<sup>7</sup>. 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

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

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 or "Sigmoidal Doses Response" curve fit on a log-log or semi-log plot and Second Order Polynomial curve fit using regular plot. There is no significant difference between the type of curve fits used in calculating samples corresponding to ATX concentration. However, the same method of analysis should be used between experiments for more accurate comparison.

## 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 all kit reagents at room temperature. Start experiment when K-PBSTB is defrosted. 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-PB-STB) 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  $\mu$ L of human serum/plasma + 245  $\mu$ L 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. Mix well and spin down. See assay note #1 for standard stability once diluted.
  - b. Prepare the 8-point ATX Standard as shown in Table 3.
6. Remove the ATX detection plate (K-5601) from plastic bag. Add 100  $\mu$ L/well of the diluted samples and prepared standards to the ATX Detection Plate (K-5601). Suggested plate layout is shown in Table 4. Cover plate with new plate seal. Incubate at room temperature for 1 hour.



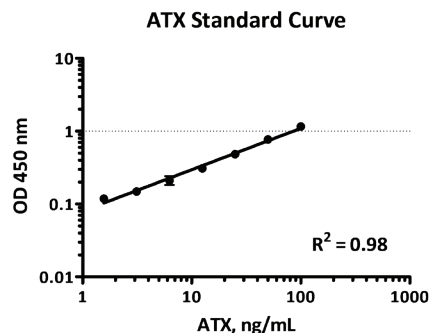
Table 3, Standard Dilutions

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

- Discard solution from plate, wash plate three times with 200  $\mu$ L 1X PBST wash buffer per well.
- Prepare the Anti-ATX Antibody by adding 12 mL 1X Diluent into the Anti-ATX Antibody (K-5603) bottle. Mix well. Add 100  $\mu$ L/well to the ATX Detection Plate (K-5601). Cover with new plate seal and incubate at room temperature for 1 hour. See assay note #1 for information on antibody stability once diluted.
- Discard solution from plate, wash plate three times with 200  $\mu$ L 1X PBST wash buffer per well.
- Prepare the Secondary Detector by mixing 0.3 mL of the Secondary Detector (K-SEC7) with 12 mL 1X Diluent. Prepare only the amount needed for the days experiment. Mix well. Add 100  $\mu$ L/well to the ATX Detection Plate (K-5601). Cover with new plate seal and incubate at room temperature for 1 hour.
- Discard solution from plate, wash plate three times with 200  $\mu$ L 1X PBST wash buffer per well.
- Add 100  $\mu$ L TMB solution per well (K-TMB1). Let blue color develop for approximately 30 minutes. Protect from light.
- Add 50  $\mu$ L 1 N H<sub>2</sub>SO<sub>4</sub> solution (K-STOPT) to each well to stop the reaction. Tap plate to mix.
- Read absorbance at 450 nm.
- 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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2. Umezu-Goto, M. et al. J Cell Biol 158 (2), 227-33 (2002).
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4. Moolenaar, W.H. J Cell Biol 158 (2), 197-9 (2002).
5. Xie, Y. et al. Cell Signal 16 (9), 975-81 (2004).
6. Parrill, A.L. et al. Anticancer Agents Med Chem 8 (8), 917-23 (2008).
7. Nakamura, K. et al. Clinica Chimica Acta 388 (1-2), 51-58 (2008).

## References (Product Publications)

1. Nakamura, R., et al. PLOS ONE, 12(6) (2017)
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
<b>Assays and Services</b>	
T-4100	Autotaxin Activity Assay Service
K-4100	Autotaxin Activity Assay
K-4200 (HTS)	Autotaxin Inhibitor Screening Kit
<b>ATX Inhibitors</b>	
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
<b>Enzymes and Substrates</b>	
E-4000	Autotaxin enzyme, active
L-2000	FS-3 (fluorogenic lysoPLD substrate)
L-2010	ATX-Red AR-2

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