Echelon Biosciences Inc.

Autotaxin (ATX) Activity Kit

K-4100 (96 tests)

Support: echelon@echelon-inc.com

Description: 96-well fluorogenic assay for quantification of ATX activity from biological samples

Materials Provided

Catalog #	Description	Amount
E-4000	Autotaxin enzyme (ATX)	1 vial
L-2000	Fluorescent autotaxin substrate (FS-3)	1 vial
K-4102	Fluorescein dye (for standard curve)	1 vial
K-4103	Lysophosphatidylcholine (LPC)	1 vial
K-4104	Buffer A (fluorescein dye suspension buffer)	1 bottle
K-4105	Buffer B (lysophosphatidylcholine suspension buffer)	1 bottle
K-4106	Buffer C (concentrated reaction buffer)	1 bottle
K-4107	Buffer D (protein stabilizer)	1 bottle
---	96 well black assay plate	1 plate
---	Plate sealer	1 seal

Quick Protocol

Prepare reagents and samples.

Add dH20 to the plate.

Add Reaction Buffer containing LPC and FS-3 to the plate.

Add dye dilutions to the plate.

Add Autotaxin enzyme dilutions to the plate.

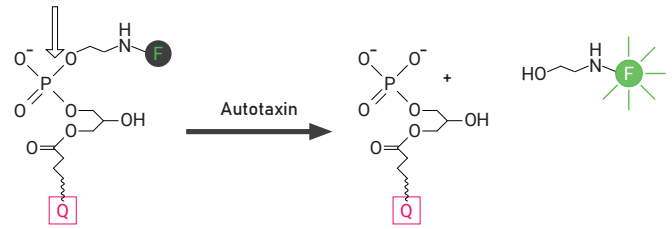
Add samples to the plate and mix for 1-3 minutes at room temperature on plate shaker.

Set plate reader for 485 ex, 528 em. and read at 37C every two minutes for 2 hours.

Analyze data.

Additional Materials Provided by User

- Fluorescence plate reader capable of exciting at 485 nm and reading at 528 nm.



Storage

Upon receipt, the kit should be stored at -20°C. Under proper storage conditions, the kit components are stable for at least six months from date of receipt. Allow the reagents to warm to room temperature before opening vials. Do not freeze thaw kit components. Once thawed use within five hours.

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com

Background

LPA is produced extracellularly largely through the lysophospholipase D (lysoPLD) activity of the enzyme autotaxin[1, 2]. Autotaxin is a 100 kDa, secreted glycoprotein that is widely expressed with high levels in the serum. Autotaxin was first isolated as the autocrine motility factor secreted from melanoma cells[3]. At that time the enzyme's lysoPLD activity had not been identified, so it was classified by homology to the ecto-nucleotide pyrophosphatase/phosphodiesterase (NPP) family of enzymes whose members hydrolyze phosphodiester bonds in various nucleotides and nucleotide derivatives. It was initially unclear how nucleotide hydrolysis could lead to the stimulation of cell motility. This mystery was solved when autotaxin was discovered to be identical to serum lysoPLD[4, 5]. Since then, the cancer-related activities of autotaxin have been attributed to the enzyme's lysoPLD activity[6, 7].

Assay Design

EBI's Autotaxin Activity Kit uses the fluorogenic autotaxin substrate FS-3. FS-3 is an LPC analogue that is conjugated with both a fluorophore and a quencher. In its native state the quencher interferes with the fluorophore's fluorescence. Once autotaxin cleaves FS-3, the fluorophore becomes liberated from the quencher, resulting in increased fluorescence[8]. This assay gives a quantitative measurement of autotaxin activity in biological samples by measuring changes in fluorescence over time. Because the assay is not end point based, it has the advantage of being less susceptible to error arising from mistiming the addition of components to different sample wells.

Assay Notes

1. This kit has been tested for the quantification of autotaxin activity in serum, plasma, ovarian ascites, and cell culture supernatant. Since serum contains autotaxin activity, cells cultured with fetal bovine serum or similar additives should use a low concentration such as 0.5% FBS and include a cell free negative control.
2. Autotaxin is a metal dependent enzyme, avoid introducing EDTA or other metal sequestering compounds when collecting biological samples. For example, do not use EDTA tubes to collect plasma samples. EDTA will block ATX activity through metal ion depletion.
3. Biological samples can contain components that interfere with fluorescence; the presence of which make measuring autotaxin activity by a fluorogenic method seem artificially low. Each sample is run in the presence of FS-3 as well as varying dilutions of the unquenched fluorophore (fluorescein) alone. The fluorescein controls allow the user to calculate a correction factor for each sample, so that the final autotaxin activity measurement is more accurate.
4. Dilutions of purified autotaxin are run to positively control for both autotaxin activity and the quantitative nature of the kit (two-fold increase in enzyme concentration lead to two-fold increase in activity measurement). The autotaxin titration is not intended to serve as a means to quantify the mass of autotaxin in biological samples. The same mass of autotaxin in different biological sample can have vastly different enzymatic activities due to the presence of other factors. Samples are run at 0.5X in order to minimally dilute these factors and more accurately reflect enzymatic activity in the biological situation
5. Both LPA and S1P inhibit autotaxin activity. Since many biological samples contain high levels of these lipids, the most accurate results will be obtained when minimal sample dilution is used. Determining if components in the sample are affecting autotaxin activity may be of interest to the researcher. In this case, a dilution series of the sample should be run in different wells and autotaxin activity should be corrected appropriately. Significant differences in corrected autotaxin activity levels between dilutions may indicate the presence of factors in the sample that alter autotaxin activity.
6. Assay is measured in ATX units. An ATX unit is defined as pM FS-3 hydrolyzed/min in 10 µM FS-3, 50 mM Tris-Cl pH 8.0, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 140 mM NaCl, 1 mg/ml Fatty Acid Free BSA, 1 mM LPC at 37°C.
7. Very low ATX activity levels can be monitored by removing LPC from the reaction buffer. However, making this change will make the assay semi-quantitative, i.e. a two-fold increase in FS-3 hydrolysis will not reflect a two-fold increase in autotaxin activity. Consequently results will no longer be in ATX units and, instead, will be measured in arbitrary units. To do so, replace the volume of LPC stock with dH2O when preparing the reaction buffer and dye buffer. For example:

   Make 3 mL of reaction buffer by adding the following components:
   - 300 µL FS-3 stock solution from step 3
   - 600 µL Buffer C (K-4106)
   - 600 µL Buffer D (K-4107)
   - 1.5 mL deionized water

   Mix by inverting 5-10 times and limit exposure to light.
8. Some organic solvents interfere with the assay. Limit to <2% final concentration of organic solvents in the assay.
9. If your plate reader is unable to regulate temperature, perform the assay at room temperature and extend the time course to four hours reading every four minutes.
10. If your plate reader is not capable of reading time courses, incubate the plate at 37°C for 20 min then read the plate as time zero. Incubate the plate at 37°C for another 70 minutes. Read the plate again as time 70 minutes. For all reaction wells subtract the value obtained time zero from the value obtained at 70 minutes. Use these values as fluorescence increase in the calculation in the third bullet of step 3 under Data Analysis.
11. If using cultured cells prepare your samples in low serum or serum free media and run a media only control the same as a sample. For best result, use a non-ATX expression cell supernatant (such as Cos-7) grown in the same media as negative control sample.
12. Avoid introducing air bubbles to assay wells. It is recommended to use dispensing pipette to transfer solution to plate to avoid introducing air bubbles. Air bubbles can affect the fluorescence reading significantly. Combined with plate shaking between readings, air bubbles may cause significant fluorescence shifts within a particular well.
13. Contact EBI and request for data analysis spreadsheet if you are using Spectra Max M2 plate reader manufactured by Molecular Devices.

Assay Protocol

Please read this entire section and Assay Notes before beginning. At least 400 µL of sample is required for the assay.

1. Place ATX enzyme (E-4000) on ice and leave other kit components at RT. Pre-set plate reader to 37°C. Equilibrate test samples at room temperature for an hour prior to use.
2. Resuspend LPC (K-4103) in 2 mL Buffer B (K-4105) to make a concentrated stock solution. Heat in a 42 °C water bath for 10 minutes. Vortex. If the solution is not clear, sonicate in water bath for 5 minutes, return to water bath for another 10 minutes, and vortex. Repeat until solution is clear.
3. Add 390 µL deioinized water to FS-3 (L-2000) to make a concen-

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com

trated stock solution. Vortex and pulse spin. Limit exposure to light.

4. Resuspend fluorescein dye standard (K-4102) in 2 mL Buffer A (K-4104) to make a concentrated stock solution. Vortex. Limit exposure to light.
5. Make 5 mL dye buffer by adding the following components:
   1 mL Buffer C (K-4106)
   1 mL Buffer D (K-4107)
   1 mL resuspended LPC from step 2
   2 mL deionized water
   Mix by inverting 5-10 times.
6. Make 3 mL of reaction buffer (RB) by adding the following components: (see Assay Note 7 if very low autotaxin activities are expected)
   300 µL FS-3 stock solution from step 3
   600 µL Buffer C (K-4106)
   600 µL Buffer D (K-4107)
   600 µL resuspended LPC from step 2
   900 µL deionized water
   Mix by inverting 5-10 times. Limit exposure to light.
7. Begin making fluorescein dilutions by labeling three 2 mL tubes "Dye 1" – "Dye 3". Prepare dilutions according to Table 1. Vortex after each addition. Limit exposure to light.
8. Label a tube "PS" (protein stabilizer) and add to it 100 µL Buffer D (K-4107) and 900 µL deionized water. Mix by inverting 5 – 10 times.
9. Pulse spin lyophilized autotaxin enzyme (E-4000). Gently add 1 mL deionized water to make a 25 nM stock solution. Mix by inverting 5-10 times. Do not vortex. Pulse spin and leave the vial at room temperature for 5 minutes to allow the enzyme to go into solution. Proceed to plate set up while waiting.
10. Add 50 µL deionized water to wells A1–D2 (Avoid introducing air bubbles. Please read Assay Note 12. See suggested plate layout below).
11. Add 50 µL reaction buffer (RB, from step 6) to wells A1- A12, E1-E12 and F1-H2.
12. Add 50 µL Dye 1 to wells B1-B12 and F3-F12, 50 µL Dye 2 to wells C1-C12 and G3-G12, 50 µL Dye 3 to wells D1-D12 and H3-H12.

Table 1

Tube	Final concentration fluorescein (pM) in well	Volume resuspended fluorescein from step 4 or previous dilution	Volume dye buffer (prepared in step 5)
Dye 1	20,000	400 µL of fluorescein from step 4	1.6 mL
Dye 2	5,000	500 µL of Dye 1	1.5 mL
Dye 3	625	200 µL of Dye 2	1.4 mL

Table 2

Tube	Final ATX concentration (nM) in well	25 nM ATX or previous dilution	PS solution
ATX 1	10	400 µL of 25 nM ATX (step 8)	100 µL
ATX 2	5	150 µL of ATX 1	150 µL
ATX 3	2.5	150 µL of ATX 2	150 µL
ATX 4	1.25	150 µL of ATX 3	150 µL

Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Spont. Hydrolysis 50 µL water 50 µL RB	Spont. Hydrolysis 50 µL water 50 µL RB	Sample 1 FS-3 50 µL Sample 1 50 µL RB	Sample 1 FS-3 50 µL Sample 1 50 µL RB	Sample 2 FS-3 50 µL Sample 2 50 µL RB	Sample 2 FS-3 50 µL Sample 2 50 µL RB	Sample 3 FS-3 50 µL Sample 3 50 µL RB	Sample 3 FS-3 50 µL Sample 3 50 µL RB	Sample 4 FS-3 50 µL Sample 4 50 µL RB	Sample 4 FS-3 50 µL Sample 4 50 µL RB	Sample 5 FS-3 50 µL Sample 5 50 µL RB	Sample 5 FS-3 50 µL Sample 5 50 µL RB
B	Dye STD1 50 µL water 50µL Dye1	Dye STD1 50 µL water 50µL Dye1	Sample 1 Control 1 50 µL Sample 1 50µL Dye1	Sample 1 Control 1 50 µL Sample 1 50µL Dye1	Sample 2 Control 1 50 µL Sample 2 50µL Dye1	Sample 2 Control 1 50 µL Sample 2 50µL Dye1	Sample 3 Control 1 50 µL Sample 3 50 µL Dye1	Sample 3 Control 1 50 µL Sample 3 50 µL Dye1	Sample 4 Control 1 50 µL Sample 4 50 µL Dye1	Sample 4 Control 1 50 µL Sample 4 50 µL Dye1	Sample 5 Control 1 50 µL Sample 5 50 µL Dye1	Sample 5 Control 1 50 µL Sample 5 50 µL Dye1
C	Dye STD2 50 µL water 50µL Dye2	Dye STD2 50 µL water 50µL Dye2	Sample 1 Control 2 50 µL Sample 1 50µL Dye2	Sample 1 Control 2 50 µL Sample 1 50µL Dye2	Sample 2 Control 2 50 µL Sample 2 50µL Dye2	Sample 2 Control 2 50 µL Sample 2 50µL Dye2	Sample 3 Control 2 50 µL Sample 3 50 µL Dye2	Sample 3 Control 2 50 µL Sample 3 50 µL Dye2	Sample 4 Control 2 50 µL Sample 4 50 µL Dye2	Sample 4 Control 2 50 µL Sample 4 50 µL Dye2	Sample 5 Control 2 50 µL Sample 5 50 µL Dye2	Sample 5 Control 2 50 µL Sample 5 50 µL Dye2
D	Dye STD3 50 µL water 50µL Dye3	Dye STD3 50 µL water 50µL Dye3	Sample 1 Control 3 50 µL Sample 1 50µL Dye3	Sample 1 Control 3 50 µL Sample 1 50µL Dye3	Sample 2 Control 3 50 µL Sample 2 50µL Dye3	Sample 2 Control 3 50 µL Sample 2 50µL Dye3	Sample 3 Control 3 50 µL Sample 3 50 µL Dye3	Sample 3 Control 3 50 µL Sample 3 50 µL Dye3	Sample 4 Control 3 50 µL Sample 4 50 µL Dye3	Sample 4 Control 3 50 µL Sample 4 50 µL Dye3	Sample 5 Control 3 50 µL Sample 5 50 µL Dye3	Sample 5 Control 3 50 µL Sample 5 50 µL Dye3
E	Autotaxin Control 1 50 µL ATX1 50 µL RB	Autotaxin Control 1 50 µL ATX1 50 µL RB	Sample 6 FS-3 50 µL Sample 6 50 µL RB	Sample 6 FS-3 50 µL Sample 6 50 µL RB	Sample 7 FS-3 50 µL Sample 7 50 µL RB	Sample 7 FS-3 50 µL Sample 7 50 µL RB	Sample 8 FS-3 50 µL Sample 8 50 µL RB	Sample 8 FS-3 50 µL Sample 8 50 µL RB	Sample 9 FS-3 50 µL Sample 9 50 µL RB	Sample 9 FS-3 50 µL Sample 9 50 µL RB	Sample 10 FS-3 50 µL Sample 10 50 µL RB	Sample 10 FS-3 50 µL Sample 10 50 µL RB
F	Autotaxin Control 2 50 µL ATX2 50 µL RB	Autotaxin Control 2 50 µL ATX2 50 µL RB	Sample 6 Control 1 50 µL Sample 6 50µL Dye1	Sample 6 Control 1 50 µL Sample 6 50µL Dye1	Sample 7 Control 1 50 µL Sample 7 50µL Dye1	Sample 7 Control 1 50 µL Sample 7 50µL Dye1	Sample 8 Control 1 50 µL Sample 8 50 µL Dye1	Sample 8 Control 1 50 µL Sample 8 50 µL Dye1	Sample 9 Control 1 50 µL Sample 9 50 µL Dye1	Sample 9 Control 1 50 µL Sample 9 50 µL Dye1	Sample 10 Control 1 50 µL Sample 10 50 µL Dye1	Sample 10 Control 1 50 µL Sample 10 50 µL Dye1
G	Autotaxin Control 3 50 µL ATX3 50 µL RB	Autotaxin Control 3 50 µL ATX3 50 µL RB	Sample 6 Control 2 50 µL Sample 6 50µL Dye2	Sample 6 Control 2 50 µL Sample 6 50µL Dye2	Sample 7 Control 2 50 µL Sample 7 50µL Dye2	Sample 7 Control 2 50 µL Sample 7 50µL Dye2	Sample 8 Control 2 50 µL Sample 8 50 µL Dye2	Sample 8 Control 2 50 µL Sample 8 50 µL Dye2	Sample 9 Control 2 50 µL Sample 9 50 µL Dye2	Sample 9 Control 2 50 µL Sample 9 50 µL Dye2	Sample 10 Control 2 50 µL Sample 10 50 µL Dye2	Sample 10 Control 2 50 µL Sample 10 50 µL Dye2
H	Autotaxin Control 4 50 µL ATX4 50 µL RB	Autotaxin Control 4 50 µL ATX4 50 µL RB	Sample 6 Control 3 50 µL Sample 6 50µL Dye3	Sample 6 Control 3 50 µL Sample 6 50µL Dye3	Sample 7 Control 3 50 µL Sample 7 50µL Dye3	Sample 7 Control 3 50 µL Sample 7 50µL Dye3	Sample 8 Control 3 50 µL Sample 8 50 µL Dye3	Sample 8 Control 3 50 µL Sample 8 50 µL Dye3	Sample 9 Control 3 50 µL Sample 9 50 µL Dye3	Sample 9 Control 3 50 µL Sample 9 50 µL Dye3	Sample 10 Control 3 50 µL Sample 10 50 µL Dye3	Sample 10 Control 3 50 µL Sample 10 50 µL Dye3

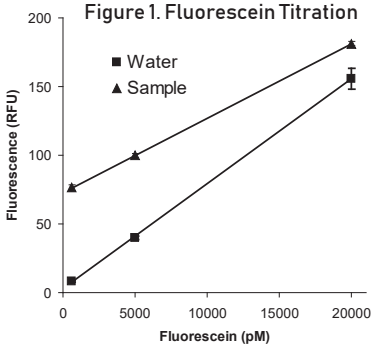
Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com

13. Begin making autotaxin dilutions by labeling four tubes "ATX 1" – "ATX 4". Add the volumes of PS (step 8) solution and autotaxin enzyme from step 9 or previous dilution listed in Table 2. Mix by gently pipeting up and down a few times before transferring solution. Do not vortex. Proceed to next step immediately.
14. Add 50 µL ATX 4 to wells H1-H2, 50 µL ATX 3 to wells G1-G2, 50 µL ATX 2 to wells F1-F2, 50 µL ATX 1 to wells E1-E2.
15. Add 50 µL Sample 1 to wells D3-A4, 50 µL Sample 2 to wells D5-A6, 50 µL Sample 3 to wells D7-A8, etc. (Refer to plate layout on the previous page.) Minimize particulate matter by centrifuging and/or filtering samples. If your samples are from cultured cells, ensure that they are serum free or contain low serum. Please see Assay Note 5 if samples are diluted. Please see Assay Note 4 if organic solvent is used.
16. Mix plate on orbital shaker for 1-3 minutes. If you do not have an orbital shaker, gently tap the plate being careful to keep all liquid in the wells.
17. Set a fluorescence plate reader for excitation at 485 nm and reading at 528 nm at 37°C. Read the plate every two minutes for two hours. See Assay Note 9 if your plate reader is not capable of regulating temperature. See Assay Note 10 if your plate reader cannot read a time course.

Data Analysis

1. Determine fluorescein standard curve in water:
   - For each data set in B1-D2, average the fluorescence values from 20 - 100 minutes.
   - Average values for each fluorescein dye standard. For example, average B1 and B2 to obtain the average fluorescein fluorescence for Dye 1 in water.
   - Plot fluorescein fluorescence versus concentration of fluorescein. The plot will have three points and the curve should resemble the plot in Figure 1; however, absolute fluorescence values may vary considerably from those in Figure 1 due to equipment differences. The dye controls will ultimately correct for equipment differences.
   - Perform linear regression on the points on the graph and record the slope of the line. You will use this slope in steps 4 and 6.

Figure 1. Fluorescein Titration



2. Determine the fluorescein standard curve for each sample.
   - For each sample control, average the fluorescence values from 20 - 100 minutes. e.g. For Sample 1 Control 1, average the fluorescence values from 20 - 100 minutes in B3-B4.
   - Plot fluorescein fluorescence versus concentration of fluorescein for each sample. Each sample will have three points (at 20,000, 5,000 and 625 pM fluorescein). Sample curves may resemble the sample trace in Figure 1. Again absolute fluorescence values will likely differ considerably from those in Figure 1.
   - Fit a line to each sample's titration data points and record the slope of each line. You will use this slope in step 5.
3. Determine the rate of fluorescence increase for data obtained from all reaction wells:
   - Plot a fluorescence versus time graph for data obtained from all wells containing reaction buffer (A1-A12, E1-H2, E3-E12).
   - Fit a line to the linear portion of each graph. Note: Often the reaction will not have come to temperature or to a steady state in the first fifteen minutes. Consequently, nonlinear fluorescence increases may be observed in this time window. Nonlinearity in the latter portion of the plot may also occur. Determining the slope between 20 and 100 minutes will usually give accurate readings.
   - Alternatively, you can use the fluorescence readings at 90 minutes as a substitute for the kinetic fitting (see assay note 10). However, this method may yield less reliable results, especially if using serum or other slightly opaque samples.
   - For each autotaxin standard or sample, average the slopes obtained from the linear fittings. e.g. Average the slopes from wells E1 and E2 to obtain the average slope for ATX 1.
4. Determine the rate of spontaneous FS-3 hydrolysis in ATX Units. Divide the averaged slopes for spontaneous hydrolysis (Wells A1 and A2, calculated in step 3) by the slope of the fluorescein fluorescence in water.
   This calculation is summarized below:

$$\text{FS-3 Hydrolysis}_{\text{Spontaneous}}\ (\text{RFU/min}) \div \text{Fluorescein Fluorescence}_{\text{Water}}\ (\text{RFU/[Fluorescein]}) = \text{FS-3 Hydrolysis}_{\text{Spontaneous}}\ (\text{ATX Units})$$

5. Convert each sample's rate of FS-3 hydrolysis into ATX units.
   - Divide the slope for each sample (calculated in step 3) by the slope of the fluorescein fluorescence in the same sample (calculated in step 2).
   - This calculation for each assay point of Sample 1 is summarized below:

$$\text{FS-3 Hydrolysis}_{\text{Sample1}}\ (\text{RFU/min}) \div \text{Fluorescein Fluorescence}_{\text{Sample1}}\ (\text{RFU/[Fluorescein]}) = \text{FS-3 Hydrolysis}_{\text{Sample1}}(\text{ATX Units})$$

6. Convert the autotaxin control samples rates of FS-3 hydrolysis into ATX units.
   - Divide the averaged slopes for each autotaxin control sample (calculated in step 3) by the slope of the fluorescein fluorescence in water.
   - This calculation for ATX1 is summarized below:

$$\text{FS-3 Hydrolysis}_{\text{ATX1}}\ (\text{RFU/min}) \div \text{Fluorescein Fluorescence}_{\text{Water}}\ (\text{RFU/[Fluorescein]}) = \text{FS-3 Hydrolysis}_{\text{ATX1}}\ (\text{ATX Units})$$

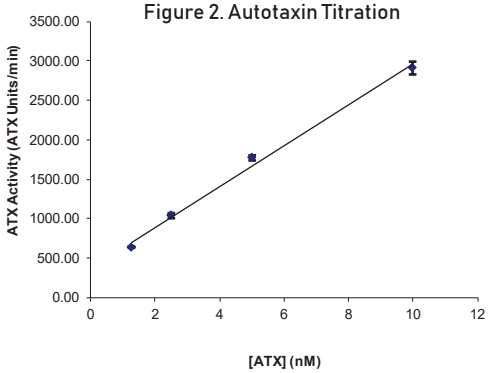
7. Correct for spontaneous FS-3 hydrolysis rate in each sample and autotaxin control. These values represent the LysoPLD activities for each sample in ATX Units (see Assay Note 6).
   - For all samples subtract the value of spontaneous hydrolysis in ATX units (calculated in step 4) from the rate calculated in step 5 for each sample.
   - For all autotaxin controls subtract the value of spontaneous FS-3 hydrolysis in ATX units (calculated in step 4) from the rate of FS-3 hydrolysis in ATX units for each autotaxin control sample (calculated in step 6).
   - This calculation for each assay point of Sample 1 is summarized below:

$$\text{FS-3 Hydrolysis}_{\text{Sample1}}\ (\text{ATX Units}) - \text{FS-3 HydrolysisS}_{\text{pontaneous}}\ (\text{ATX Units}) = \text{LysoPLD Rate}_{\text{Sample1}}\ (\text{ATX Units})$$

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com

8. Plot LysoPLD rate versus autotaxin concentration for autotaxin titration. See Table 2 for autotaxin concentrations. The plot should resemble Figure 2. This curve serves to positively control for both autotaxin activity and the quantitative nature of the kit (two-fold increase in enzyme mass leads to two-fold increase in activity measurement). The autotaxin titration is not intended to serve as a means to quantify the mass of autotaxin in biological samples. The same mass of autotaxin in different biological sample can have vastly different enzymatic activities due to the presence of other factors. Samples are run at 0.5x in order to minimally dilute these factors and more accurately reflect enzymatic activity in the biological situation (see Assay Notes 4 and 5).

Figure 2. Autotaxin Titration



Background References

1. Tokumura, A. et al. Biol Reprod 67 (5), 1386-92 (2002).
2. Aoki, J. et al. J Biol Chem 277 (50), 48737-44 (2002).
3. Stracke, M.L. et al. J Biol Chem 267 (4), 2524-9 (1992).
4. Clair, T. et al. Cancer Res 63 (17), 5446-53 (2003).
5. Umezu-Goto, M. et al. J Cell Biol 158 (2), 227-33 (2002).
6. Moolenaar, W.H. J Cell Biol 158 (2), 197-9 (2002).
7. Xie, Y. et al. Cell Signal 16 (9), 975-81 (2004).
8. Ferguson, C.G. et al. Org Lett 8 (10), 2023-6 (2006).
9. Hammack, B.N. et al. Mult Scler 10 (3), 245-60 (2004).
10. Baker, D.L. et al. J Biol Chem 281 (32), 22786-93 (2006).
11. Nam, S.W. et al. Oncogene 19 (2), 241-7 (2000).
12. Croset, M. et al. Biochem J 345 Pt 1, 61-7 (2000).

Product References

1. Cochrane W. et al. ACS Comb Sci 21(5):425-35 (2019).
2. Nsaibia M.J. et al. J Int Med 277 280:509-17 (2016).
3. Shelton E.L. et al. PLoS ONE 8(7):e69712 (2013).
4. Xia Q. et al. Acta Pharmacol Sin 32(8):1055 (2011).
5. Wu J.M. et al. Mol Cancer 9:71 (2010).

Related Products

Catalog #	Products
Assays and Services	
T-2800S	LPA ELISA Assay Service
T-4100	Autotaxin Activity Assay Service
T-4200	Autotaxin Inhibitor Screening Service
K-2800S	LPA ELISA Kit
K-4200	Autotaxin Inhibitor Screening Kit
K-5600	Autotaxin Sandwich ELISA
T-5600	ATX Sandwich ELISA Assay Service
ATX Inhibitors	
B-0701	HA130
B-0702	PF-8380
L-3282	S32826
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

Please visit our website at www.echelon-inc.com for more enzyme and lipid products.

Echelon Biosciences Inc.
Ph: 801-588-0455
Fax: 801-588-0497
Echelon-inc.com