

Autotaxin Inhibitor Screening Assay Kit

Item No. 700580



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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results store kit at -80°C or remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
700581	Autotaxin Assay Buffer (10X)	1 vial/5 ml	-20°C
700582	Autotaxin (human recombinant) Assay Reagent	2 vials/60 µl	-80°C
700583	Autotaxin Substrate	2 vials/lyophilized	-20°C
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	Room temperature
400012	96-Well Cover Sheet	1 cover	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-415 nm
2. Adjustable pipettes and a repeating pipettor
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Autotaxin (ATX, Ectonucleotide Pyrophosphatase/Phosphodiesterase-2, ENPP-2, Lysophospholipase D) is a secreted lysophospholipase D that catalyzes the hydrolysis of lysophosphatidylcholine (LPC) to generate lysophosphatidic acid (LPA). LPA is a lipid mediator that activates G protein-coupled receptors and induces a variety of biological responses, such as neurogenesis, angiogenesis, smooth-muscle contraction, platelet aggregation, and wound healing.¹ ATX-LPA signaling is involved in a range of pathologies including tumor progression and inflammation.² ATX is the only factor known to exhibit lysophospholipase D activity in serum. ATX is proteolytically processed by cleavage of a 35 amino acid signal peptide and then secreted as a mature protein.³ N-glycosylation of ATX at Asn53 and Asn410 is essential for activity and secretion of ATX from adipocytes.¹

ATX levels are elevated in the cerebrospinal fluid of multiple sclerosis patients and in the serum of liver fibrosis patients.^{4,5} ATX is widely known for its involvement in cancer.⁶ A gene chip analysis found that ATX is among the forty most upregulated genes in highly metastatic cancers.⁷

About This Assay

Cayman's Autotaxin Inhibitor Screening Assay provides a convenient method for screening human ATX inhibitors. ATX cleaves *bis*-(*p*-nitrophenyl) phosphate liberating *p*-nitrophenol, a yellow product that is measured at 405-415 nm.

PRE-ASSAY PREPARATION

Reagent Preparation

1. Autotaxin Assay Buffer (10X) - (Item No. 700581)

The vial contains 5 ml of 500 mM Tris-HCl, pH 9.0, containing 50 mM CaCl_2 . Dilute the contents of the Assay Buffer concentrate vial with 45 ml of HPLC-grade water. This final Assay Buffer (50 mM Tris-HCl, pH 9.0, containing 5 mM CaCl_2) is used in the assay and for diluting reagents. When stored at 4°C, this diluted Assay Buffer is stable for one month.

2. Autotaxin (human recombinant) Assay Reagent - (Item No. 700582)

Each vial contains 60 μl of human recombinant Autotaxin (ATX). Thaw the enzyme on ice, add 540 μl of diluted Assay Buffer to the vial, and vortex. The diluted enzyme is stable for four hours on ice. One vial of enzyme is sufficient to assay 60 wells. Use the additional vial if assaying the entire plate.

3. Autotaxin Substrate - (Item No. 700583)

Each vial contains a lyophilized powder of *bis*-(*p*-nitrophenyl) phosphate (BNPP). Reconstitute the contents of the vial with 1.2 ml of diluted Assay Buffer. One vial of Substrate is sufficient reagent to assay 60 wells. Reconstitute the additional vial if assaying the entire plate. The reconstituted Substrate is stable for two weeks at -20°C. *NOTE: The final concentration of Substrate in the assay as described is 3 mM. This concentration may be reduced with diluted Assay Buffer at the user's discretion. The K_m for the Substrate is 2.64 mM.*

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as 100% Initial Activity wells and three wells designated as background wells. We suggest that each test compound be assayed in triplicate and that you record the contents of each well on the template sheet provided (see page 15). A typical layout of samples and inhibitors to be measured in triplicate is shown in Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells

A - 100% Initial Activity Wells

1-30 - Test Compound Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 190 μ l in all the wells.
- All reagents except the enzyme must be equilibrated to room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- We recommend assaying test compounds in triplicate, but it is the user's discretion to do so.
- The assay is performed at 37°C.
- Monitor the absorbance at 405-415 nm.

Performing the Assay

1. **100% Initial Activity Wells** - add 150 μ l of diluted Assay Buffer, 10 μ l of ATX, and 10 μ l of solvent (same solvent used to dissolve the inhibitor) to three wells.
2. **Background Wells** - add 160 μ l of diluted Assay Buffer and 10 μ l of solvent (same solvent used to dissolve the inhibitor) to three wells.
3. **Test Compound Wells** - add 150 μ l of diluted Assay Buffer, 10 μ l of ATX, and 10 μ l of Test Compound* to three wells.

	Assay Buffer	ATX	Solvent	Test Compound
100% Initial Activity	150 μ l	10 μ l	10 μ l	-
Background	160 μ l	-	10 μ l	-
Test Compound	150 μ l	10 μ l	-	10 μ l

4. Initiate the reactions by adding 20 μ l of Autotaxin Substrate to the 100% Initial Activity, Background, and Test Compound wells.
5. Cover the plate with the plate cover and incubate for thirty minutes at 37°C.
6. Remove the plate cover and read the absorbance at a wavelength between 405-415 nm.

*Test Compounds can be dissolved in Assay Buffer, methanol, or ethanol and should be added to the assay in a final volume of 10 μ l. In the event that an appropriate concentration of the Test Compound is completely unknown, we recommend that several dilutions of the Test Compound be made.

ANALYSIS

Calculations

1. Determine the average absorbance of the background, 100% initial activity (IA), and Test Compound (TC) wells.
2. Subtract the average absorbance of the background wells from the average absorbance of the 100% IA and TC wells.
3. Determine the percent inhibition or percent Initial Activity for each TC using one of the following equations.

$$\% \text{ Inhibition} = \left[\frac{\text{IA} - \text{TC Activity}}{\text{IA}} \right] \times 100$$

$$\% \text{ Initial Activity} = \frac{\text{TC Activity}}{\text{IA}} \times 100$$

4. Graph the percent inhibition or percent initial activity as a function of the TC concentration to determine the IC_{50} value (concentration at which there was 50% inhibition). Inhibition of human recombinant ATX by S32826 (Item No. 13664), a potent and selective inhibitor of ATX, is shown in Figure 2 (see page 11).⁸

Performance Characteristics

Precision:

When a series of sixteen ATX measurements were performed on the same day, the intra-assay coefficient of variation was 3.7%. When a series of sixteen ATX measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 4.5%.

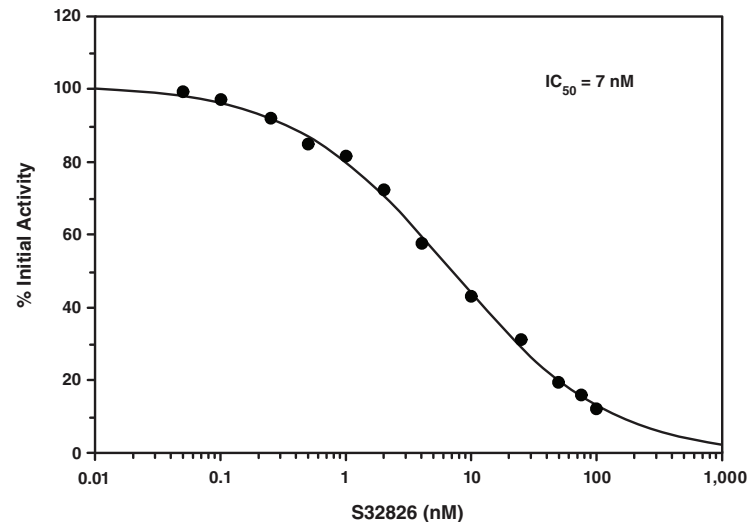


Figure 2. Inhibition of human recombinant ATX by S32826

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No absorbance detected above background in the Test Compound wells	A. Enzyme was not added to the well(s) B. Test compound concentration was too high and resulted in complete inhibition of the enzyme activity	A. Make sure to add all of the components to the wells B. Reduce the concentration of the test compound and re-assay
No inhibition was seen with test compound	A. The test compound concentration was not high enough B. The test compound is not an inhibitor of the enzyme	Increase the test compound concentration and re-assay

References

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4. Hammack, B.N., Fung, K.Y., Hunsucker, S.W., *et al.* Proteomic analysis of multiple sclerosis cerebrospinal fluid. *Mult. Scler.* **10(3)**, 245-60 (2004).
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8. Ferry, G., Moulharat, N., Pradère, J.-P., *et al.* S32826, a nanomolar inhibitor of autotaxin: Discovery, synthesis and applications as a pharmacological tool. *J. Pharmacol. Exp. Ther.* **327**, 809-819 (2008).

Related Products

Autotaxin (human recombinant) - Item No. 10803
 Lysophospholipase D Polyclonal Antibody - Item No. 10005375
 Phospholipase D Assay Kit - Item No. 700590
 S32826 - Item No. 13664
 Total Phosphatidic Acid Fluorometric Assay Kit - Item No. 700240

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s **exclusive remedy** and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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NOTES

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