Autotaxin Inhibitor Screening Assay Kit

Item No. 700580

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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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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
700584	HA-155 Positive Control Inhibitor	1 vial/100 µl	-20°C
400014	96-Well Solid Plate (Colorimetric Assay)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

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 RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

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

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 mix gently. 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.*

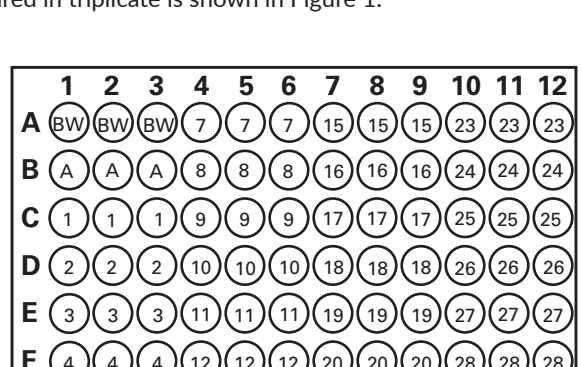
4. **HA-155 Positive Control Inhibitor - (Item No. 700584)**

   This vial contains a 2 mM solution of HA-155 in DMSO. For use in Positive Control Inhibitor wells, dilute in Assay Buffer to 0.019 mM; 10 µl of this will give a final concentration of 1 µM in the well.

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 19). A typical layout of samples and inhibitors to be measured in triplicate is shown in Figure 1.



BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a repeating pipettor or multichannel pipette 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 µl 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 µl of diluted Assay Buffer, 10 µl of ATX, and 10 µl of vehicle (same solvent* used to dissolve the inhibitor) to three wells.
2. **Positive Control Inhibitor Wells** - add 150 µl of diluted Assay Buffer, 10 µl of ATX, and 10 µl of HA-155 Positive Control Inhibitor to three wells. The final concentration of HA-155 in the 100% Inhibition wells is 1 µM.
3. **Background Wells** - add 160 µl of diluted Assay Buffer and 10 µl of solvent (same solvent used to dissolve the inhibitor) to three wells.
4. **Test Compound Wells** - add 150 µl of diluted Assay Buffer, 10 µl of ATX, and 10 µl of Test Compound** to three wells.

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

Table 1. Test Compound Wells

5. Initiate the reactions by adding 20 µl of Autotaxin Substrate to the 100% Initial Activity, Background, and Test Compound wells.
6. Cover the plate with the plate cover and incubate for 30 minutes at 37°C.
7. Remove the plate cover and read the absorbance at a wavelength between 405-415 nm.

*This assay is sensitive to solvent concentrations. Solvent concentrations in vehicle prep should be kept below 1%. Table 2, on page 13, lists standard solvents and their effect on Initial Activity when added neat to the assay wells (5.3% solvent in-well).

**Test Compounds can be dissolved in Assay Buffer, DMSO, methanol, or ethanol and should be added to the assay in a final volume of 10 µl. 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.

Solvent	100% Initial Activity	% Activity Relative to Assay Buffer
Assay Buffer	1.0653	100%
DMSO	0.7247	68%
Methanol	0.6773	64%
Ethanol	0.5025	47%

Table 2. Solvent Tolerance

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.

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

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

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 HA-155 (Item No. 11034), a potent and selective inhibitor of ATX, is shown in Figure 2 (see page 15).[8]

Performance Characteristics

Precision:

When a series of 16 ATX measurements were performed on the same day, the intra-assay coefficient of variation was 3.7%. When a series of 16 ATX measurements were performed on six different days under the same experimental conditions, the inter-assay coefficient of variation was 4.5%.

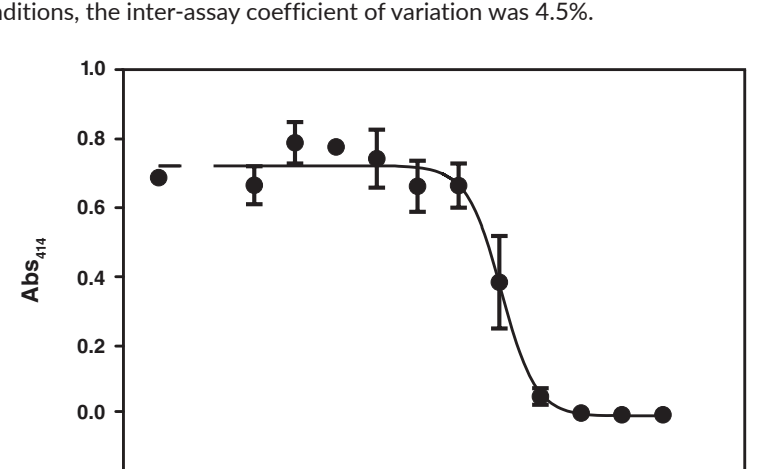


Figure 2. Inhibition of human recombinant ATX by HA-155. The IC_{50} range for a typical HA-155 inhibition curve using this kit should fall between 5.7 and 10.2 nM. "Veh." represents compound vehicle control.

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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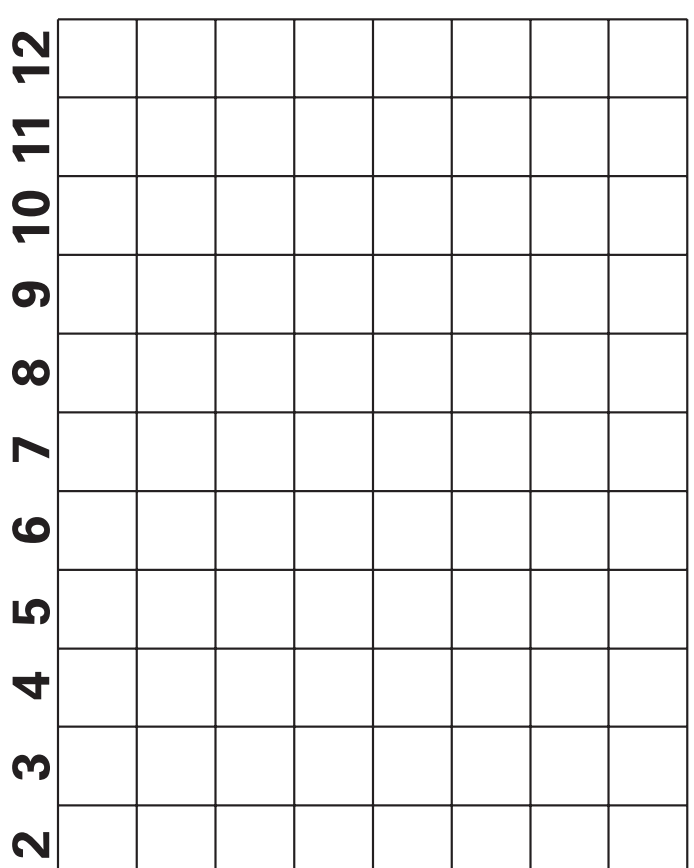
6. Baker, D.L., Fujiwara, Y., Pigg, K.R., *et al.* Carba analogs of cyclic phosphatidic acid are selective inhibitors of autotaxin and cancer cell invasion and metastasis. *J. Biol. Chem.* **281(32)**, 22786-22793 (2006).
7. Euer, N., Schwirzke, M., Evtimova, V., *et al.* Identification of genes associated with metastasis of mammary carcinoma in metastatic *versus* non-metastatic cell lines. *Anticancer Res.* **22**, 733-740 (2011).
8. Hausmann, J., Kamtekar, S., Christodoulou, E., *et al.* Structural basis of substrate discrimination and integrin binding by autotaxin. *Nature Structural & Molecular Biology* **18(2)**, 198-204 (2011).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.