AMP+ MaxSpec® Kit

Item No. 710000

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	15 Tests Quantity/Size	50 Tests Quantity/Size	100 Tests Quantity/Size
710001	AMP+ Derivatizing Reagent - 20 mM in MeCN	1 vial /450 µl	1 vial/1.5 ml	1 vial/3 ml
710002	HOBt - 20 mM in 99:1 MeCN:DMF	1 vial/300 µl	1 vial/1 ml	1 vial/1.5 ml
710003	EDC - 640 mM in water	1 vial/3 ml	1 vial/3 ml	1 vial/3 ml
710004	4:1 MeCN/DMF	1 vial/3 ml	1 vial/3 ml	1 vial/3 ml
710006	Autosampler Glass Vial Inserts (300 µl)	15	50	100

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.

Limitations

This kit should not be used to study stearic acid or palmitic acid. We have observed the presence of these two acids in the kit upon following the derivatization procedure outlined in the booklet. Fatty acid contamination such as this has been reported in the literature. We suggest following alternate published procedures for the analysis of palmitic acid and stearic acid.[1,2]

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 if stored as directed on page 3 and used before the expiration date indicated on the outside of the box.

All reagents in the AMP$^+$ MaxSpec® Kit may be used as provided. Reagents should be handled cold and resealed immediately after use to prevent solvent evaporation. Frozen reagents should be thawed completely prior to use to ensure uniform concentration. The entire contents of the Kit should be stored at -20°C when not in use.

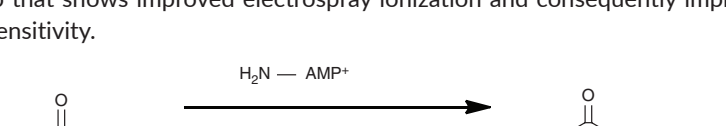
Materials Needed But Not Supplied

1. Adjustable pipettes - 100 µl capacity
2. Standard 2 ml autosampler vials with either screw or crimp caps
3. Oven or heating bath controlled at 60°C

INTRODUCTION

About This Assay

It is well documented that mass spectrometry (MS) sensitivity may be improved for certain classes of compounds *via* formation of charged chemical derivatives.[3-7] Many eicosanoids and lipids have a free carboxylic acid group that typically requires a weak acid mobile phase to ensure retention by HPLC. Unfortunately, the presence of the acid in the mobile phase also suppresses anion formation and often leads to poor electrospray ionization and poor MS sensitivity. Cayman's AMP$^+$ MaxSpec® Kit contains a positively charged reagent (AMP$^+$) that can be coupled quantitatively to free carboxylic acids through an amide bond linkage (Scheme 1). The resulting derivatized product thus contains a positively charged group that shows improved electrospray ionization and consequently improved MS sensitivity.



Scheme 1.

Another equally important benefit of this reagent is the stability of the derivative group to MS fragmentation. Reagent-specific fragmentation, or fragmentation on the reagent side of the derivatized species, is not desirable as co-migrating, isobaric analytes will give rise to identical fragment ions. Cayman's derivatization reagent is stable to MS/MS fragmentation and so isobaric derivatives undergo more traditional compound-specific fragmentation to give unique fragment ions. **Figure 1**, below, demonstrates this for derivatized PGF$_{2\alpha}$. Note that MS/MS fragmentation occurs on the bottom chain rather than on the derivative end.

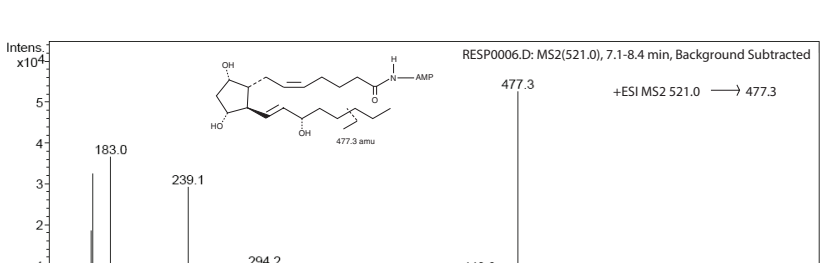


Figure 1. MS/MS spectra of AMP$^+$-derivatized PGF$_{2\alpha}$

Sample Preparation

NOTE: The kit has not been tested with unextracted biological matrices.

Standard sample preparation such as Solid-Phase Extraction (SPE) or Liquid-Liquid Extraction (LLE) is recommended prior to derivatization.

General Information

- The structure of the AMP^+ derivatizing reagent is proprietary. However, its molecular formula is $C_{12}H_{13}N_2^+$.
- Calculation of derivatized product mass:

Derivatized Product m/z = Product m/z - H_2O (18.01 amu) + $C_{12}H_{13}N_2^+$ (185.11 amu)

e.g., $PGF_{2\alpha}$ - H_2O + AMP+ ($C_{12}H_{13}N_2^+$) = $PGF_{2\alpha}$-AMP^+
354.24 amu - 18.01 amu + 185.11 amu = 521.34 amu

Performing the Assay

1. Add 8 µl of sample to a standard autosampler vial and dry the sample under nitrogen.
2. Add 20 µl of cold 4:1 MeCN/DMF solvent.
3. Add 20 µl of the cold EDC solution.
4. Add 10 µl of the HOBt solution.
5. Add 30 µl of the AMP^+ solution.
6. Vortex and transfer the sample to a 200 µl vial insert using a 100 µl adjustable pipettor. Place the insert in an autosampler vial and seal.
7. Heat the vial at 60°C for 30 minutes and then allow the vial to cool to room temperature.
8. The sample can be analyzed immediately or stored at -20°C until the analysis.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values	A. Poor pipetting/technique B. Old reagents C. Air bubble at bottom of insert	A. Use proper pipetting technique B. Replace reagents C. Vortex vial with insert to remove air bubbles
No derivatized product was detected in the sample	A. Mixture was not prepared correctly B. Compound may not react under the derivatization conditions or maybe unstable	A. Prepare fresh sample and repeat experiment B. Temperature and/or reaction time may be modified to improve reaction kinetics or reduce sample degradation as needed
Derivatized product does not provide improved sensitivity	A. Compounds that already ionize extremely well in underivatized form may not experience dramatic benefit B. Unstable compound C. Matrix ion suppression effects	A. Derivatization may not be necessary B. Test the feasibility of the derivatization with standards prior to analyzing matrix samples C. Include internal standard controls to account for matrix effects
Derivatizing reagent precipitates upon storage	Reagent precipitation is common; this has been investigated and the reagent may be redissolved without affecting kit function	Warm the reagent solution to room temperature or slightly above and vortex the solution until the solution is homogenous (approx. 5-10 minutes)

References

1. Yao, C.-H., Liu, G.-Y., Yang, K., *et al.* Inaccurate quantitation of palmitate in metabolomics and isotope tracer studies due to plastics. *Metabolomics* **12**, 143 (2016).
2. Cavonius, L.R. and Carlsson, N.-G. Fatty acid contaminations originating from commercially available solid phase-extraction columns. *Chem. Sci. Rev. Lett.* **4(16)**, 1206-1209 (2015).
3. Higashi, T. and Shimada, K. Derivatization of neutral steroids to enhance their detection characteristics in liquid chromatography-mass spectrometry. *Anal. Bioanal. Chem.* **378(4)**, 875-882 (2004).
4. Hong, H. and Wang, Y. Derivatization with Girard reagent T combined with LC-MS/MS for the sensitive detection of 5-formyl-2'-deoxyuridine in cellular DNA. *Anal. Chem.* **79(1)**, 322-326 (2007).
5. Higashi, T., Nishio, T., Hayashi, N., *et al.* Alternative procedure for charged derivatization to enhance detection responses of steroids in electrospray ionization-MS. *Chem. Pharm. Bull. (Tokyo)* **55(4)**, 662-665 (2007).
6. Woo, H.-K., Go, E.P., Hoang, L., *et al.* Phosphonium labeling for increasing metabolomic coverage of neutral lipids using electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **23(12)**, 1849-1855 (2009).
7. Eggink, M., Wijtmans, M., Kretschmer, A., *et al.* Targeted LC-MS derivatization for aldehydes and carboxylic acids with a new derivatization agent 4-APEBA. *Anal. Bioanal. Chem.* **397(2)**, 665-675 (2010).

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.