Lysosomal Acid Lipase Activity MaxSpec® Assay Kit

Item No. 24854

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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	Quantity/Size	Nominal Concentration*	Storage
24830	LAL Activity MaxSpec® Assay Substrate	600 µl/ampule	1 mg/ml in methyl acetate	-20°C
24831	LAL Activity MaxSpec® Assay Product	600 µl/ampule	100 µg/ml in ethanol	-20°C
24832	LAL Activity MaxSpec® Assay Internal Standard	600 µl/ampule	10 µg/ml in ethanol	-20°C
28361	Cardiolipin (bovine heart) Solution	400 µl/ampule	5 mg/ml in ethanol	-20°C
28362	LAL Activity Assay Buffer	3 ml/vial	0.1 M	-20°C

*_**Batch specific**_ concentrations can be found on individual Certificates of Analysis

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: 734-971-3640

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 if stored as directed in the Materials Supplied section and used before the expiration date indicated on the outside of the box. The LAL Activity MaxSpec® Assay Substrate (Item No. 24830) may precipitate out of solution upon cold storage. If this occurs, warm the ampule to room temperature and sonicate or vortex until the solution is completely homogenous before use.

Materials Needed But Not Supplied

1. Deionized water
2. HPLC autosampler vials (such as Phenomenex p/n AR0-9921-13-C)
3. Acetonitrile, LC-MS grade or equivalent
4. Isopropanol, LC-MS grade or equivalent

Equipment

1. Oven or incubator controlled at 70°C
2. Pipettors (10 µl, 200 µl, and 1,000 µl)
3. LC-MS (a Waters ACQUITY UPLC and Xevo TQ-S micro Triple Quadrupole Mass Spectrometer were used to develop the procedures outlined in this kit)
4. Microcentrifuge (optional)
5. Vacuum SPE manifold (optional)
6. SpeedVac concentrator (optional)

INTRODUCTION

Background

Lysosomal acid lipase (LAL) is a lysosomal enzyme that hydrolyzes cholesteryl esters and triglycerides to produce cholesterol, glycerol, and free fatty acids.[1] LAL deficiency is due to mutations in the LAL gene, *LIPA*, that lead to decreases in LAL activity. Wolman's disease is a severe form of LAL deficiency that begins in infancy and is characterized by a nearly complete or complete lack of LAL activity resulting in gastrointestinal disorders, hepatomegaly, and failure to thrive, leading to hypercholesterolemia and fatality within months without treatment. Cholesterol ester storage disorder is a less severe form of LAL deficiency in which LAL activity is reduced but not abolished. It presents later in life and is characterized by gastrointestinal disturbances, dyslipidemia, hepatomegaly, and impaired liver function. Research toward development of methods to detect deficiency in this enzyme has become an important goal in diagnosing and treating individuals with this disorder.

About This Assay

Cayman's LAL Activity MaxSpec® Assay Kit includes the necessary reagents to quantify LAL enzyme activity in dried blood spots. The LAL Activity MaxSpec® Assay Substrate (P-PMHC) has been designed to function as a highly selective substrate for LAL, which hydrolyzes the fatty acyl group of P-PMHC resulting in the formation of the LAL Activity MaxSpec® Assay Product 4-propyl-8-methyl-7-hydroxycoumarin (PMHC).[2] A schematic of this process is shown in Figure 1, below. Using the LAL Activity MaxSpec® Assay Kit, LAL activity may be quantified directly by fluorometry or by LC-MS in combination with the LAL Activity MaxSpec® Assay Internal Standard.

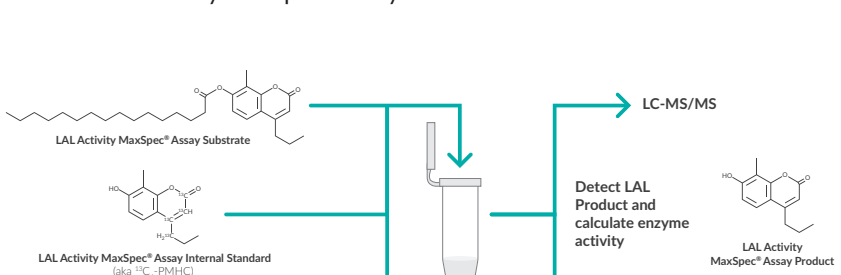

Figure 1. LAL Activity MaxSpec® Assay Kit Workflow

ASSAY PROTOCOL

The procedure for quantifying LAL enzyme activity in dried blood spot punches has been described in detail previously.[2]

NOTE: The assay buffer may be prepared in advance and stored at 4°C for several months.

Substrate Preparation

NOTE: The LAL Activity MaxSpec® Assay Substrate may precipitate out of solution upon cold storage. If this occurs, warm the ampule to room temperature and sonicate or vortex until the solution is completely homogenous before use.

Using the batch specific concentration from the Certificate of Analysis (CofA) and the formula below, add 0.9 µmol of LAL Activity MaxSpec® Assay Substrate (Item No. 24830) to a 5-10 ml glass vial. Add 0.0151 µmol of LAL Activity MaxSpec® Assay Internal Standard (Item No. 24832) and evaporate the solvent under a gentle stream of nitrogen.

Substrate:

Volume to add (ml) = [0.9 µmol x 0.457 (mg/µmol)] / ***batch specific*** concentration from CofA (mg/ml)

Internal Standard:

Volume to add (ml) = [0.0151 µmol x 0.222 (mg/µmol)] / ***batch specific*** concentration from CofA (µg/ml)

NOTE: If detecting LAL enzyme activity fluorometrically, do not add internal standard to the substrate.

Assay Mix Preparation

Add 0.3 ml of the included bovine heart cardiolipin solution in ethanol to the dried vial to resuspend the substrate and internal standard. The substrate and internal standard should be dissolved completely in the cardiolipin solution before adding the LAL Activity MaxSpec® Assay Buffer (Item No. 28362). Add 2.7 ml of assay buffer and mix thoroughly. This provides the assay mix containing 0.3 mM substrate and 5.03 µM internal standard. Internal standard addition is not required for fluorometric assays.

NOTE: The substrate and internal standard solution in ethanolic bovine cardiolipin may be prepared and stored at -20°C for several months; however, freeze thaw cycles of more than 2-3 should be avoided.

NOTE: Prepare the assay mix immediately prior to use. This protocol provides sufficient assay mix for up to 100 tests.

Performing the Assay

1. Add 3 mm dried blood spot (DBS) punches and 200 µl deionized water to 1.5 ml polypropylene tubes.
2. Incubate tubes with gentle shaking for 1 hour at room temperature.
3. Vortex briefly and add 10 µl to a single well of a deep well 96-well plate.

   NOTE: If detecting LAL enzyme activity fluorometrically, do not add internal standard to the substrate when preparing the assay mix.
4. Add 30 µl of assay mix and centrifuge the plate at 3,000 x g for 5 minutes to ensure all liquid is at the well bottom.
5. Seal the plate with a polytetrafluoroethylene (PTFE) mat and incubate at 37°C on an orbital shaker at 400 rpm for 3 hours.

LC-MS/MS Assay Readout

1. Quench the reaction by adding 80 µl of deionized water followed by 400 µl of HPLC-grade ethyl acetate.
2. Mix the contents by pipetting 10 times and centrifuge at 3,000 x g for 5 minutes at room temperature.
3. Transfer 120 µl of the upper organic layer to a shallow well 96-well plate and evaporate the solvent with a gentle stream of nitrogen at room temperature.
4. Add 200 µl of a 1:1 solution of water:methanol and mix by pipetting a few times.
5. Seal the plate with foil and place in an autosampler at 8°C for LC-MS/MS analysis.

Fluorometric Assay Readout

1. Quench reaction with 200 µl of 1:1 water/methanol and mix by pipetting up and down approximately 10 times.
2. Transfer 150 µl of the well contents to a black flat-bottomed 96-well plate and immediately read in a fluorometer with an excitation wavelength of 355 nm, emission wavelength of 460 nm, and excitation time of 0.1 seconds. Convert the fluorometer reading to micromoles of product by generating a standard curve. See published protocol for additional details.[2]

LC-MS/MS Method

LC-MS/MS Analysis

A Waters ACQUITY UPLC I-Class and Xevo TQ-S micro Triple Quadrupole Mass Spectrometer were used for LC-MS/MS analysis.

HPLC Settings

A Waters ACQUITY UPLC 2.1 x 50 mm, 1.7 µm C18 column, with a 1.7 µm guard column at a 0.8 ml/min flow rate at room temperature was used for separation of the components. The injection volume was 10 µl.

Mobile Phase A: 70:30 water:acetonitrile with 0.1% formic acid

Mobile Phase B: 50:50 acetonitrile:isopropanol with 0.1% formic acid

HPLC Gradient Program

Time (minutes)	%A	%B
0	99	1
1	30	70
1.1	0	100
1.6	0	100
1.7	99	1
2.5	99	1

Mass Spectrometry Settings

Parameter	Value
Capillary Voltage	2.95 kV
Extractor Voltage	3 V
Desolvation Temperature	450°C
Source Temperature	150°C
Desolvation Gas Flow	850 L/hr
Cone Gas Flow	30 L/hr
Collision Gas Flow	0.15 ml/min
LM Resolution 1	2.82
HM Resolution 1	14.92
Ion Energy 1	0.8
LM Resolution 2	2.88
HM Resolution 2	14.7
Ion Energy 2	1.1
Aperture	0.1
Entrance	0.5
Exit	0.5
Gain	1

Multiple Reaction Monitoring Parameters*

Compound	Parent (m/z)	Daughter (m/z)	Dwell (s)	Cone (V)	Collision (V)
LAL Activity MaxSpec® Assay Product	219.1	190	0.05	40	22
LAL Activity MaxSpec® Assay Internal Standard	223.1	194	0.05	40	22

*All data for activity of substrate was determined from the ratio of the traces of product over internal standard.

ANALYSIS

Calculations

Enzyme activity (A_e) may be calculated from the MS/MS data using the following formula:

A_e (mmol h^{-1} L^{-1}) = [(product area/internal standard area) x internal standard assay concentration (µM) x assay volume (µl) / [(incubation time (h) x estimated DBS volume (L)]

The estimated volume in a 3 mm DBS punch is 3.1 µl based on a previous publication.[3]

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No peaks observed for product or internal standard	Materials were not added to assay; MS conditions require further optimization; HPLC conditions are not equilibrated	Test LC and MS for proper performance: run performance test using provided materials to ensure they chromatograph and are detectable; prepare new mobile phases
Poor precision	Loss of analyte(s) on well-plate walls	Be sure to centrifuge all liquid to bottom of plates during reaction workup
Poor accuracy	Aging buffer or cardiolipin/ substrate solution; poor pipetting; using nominal analyte concentrations rather than batch specific concentrations	Prepare new solutions; verify accuracy of pipetting; see CofA for batch specific concentrations, which are often very close to or exactly the same as the nominal concentrations shown on the vials; however, be sure to verify the exact concentrations of the solutions for highest accuracy

References

1. Pericleous, M., Kelly, C., Wang, T., *et al. Lancet Gastroenterol. Hepatol.* **2(9)**, 670-679 (2017).
2. Masi, S., Chennamaneni, N., Turecek, F., *et al. Clin. Chem.* **64(4)**, (2018).
3. Spacil, Z., Tatipaka, H., Barcenas, M., *et al. Clin. Chem.* **59(3)**, 501-511 (2013).

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.