Biotinidase Activity MaxSpec® Assay Kit

Item No. 27544

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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 Temp.
27541	Biotinidase Activity MaxSpec® Assay Substrate	0.35 ml/ampule	5 mM in dimethyl formamide (DMF)	-20°C
24602	Biotinidase Activity Assay Product	1 mg/vial	A neat solid	-20°C
27542	Biotinidase Activity MaxSpec® Assay Internal Standard	0.35 ml/ampule	25 µM in methanol	-20°C
27543	Biotinidase Activity Assay Buffer	5 ml/bottle	0.5 M Tris + 5 mg/ml sodium taurocholate	-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 Biotinidase Activity MaxSpec® Assay Substrate (Item No. 27541) 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
5. Glacial acetic acid

Equipment

1. Oven or incubator controlled at 70°C
2. Pipettors (10, 200, 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

Biotinidase is a peptidyl hydrolase that cleaves biocytin and biotinylated peptides *in vivo* to generate free biotin, an essential coenzyme for certain carboxylases involved in fatty acid, amino acid, and glucose metabolism.[1,2] Early-onset biotinidase deficiency is due to deletion, insertion, substitution, or missense mutations in the biotinidase gene, *BTD*, that affect the activity of multiple carboxylases. This profound biotinidase deficiency results in seizures, ataxia, sensorineural hearing loss or vision loss, eczema, and respiratory disruptions without treatment. Partial biotinidase deficiency is due to a substitution mutation in the *BTD* gene resulting in a 70-90% reduction in biotinidase activity leading to symptom manifestation that may not occur until adolescence and occurs only under biological stress. Many symptoms resolve with treatment, but retinal degeneration, sensorineural hearing loss, and developmental delays cannot be reversed. Early detection of biotinidase deficiency is imperative to avoid permanent damage.

About This Assay

Cayman's Biotinidase Activity MaxSpec® Assay Kit includes the necessary reagents to quantify biotinidase enzyme activity in dried blood spots. The Biotinidase Activity MaxSpec® Assay Substrate has been designed to function as a highly selective substrate for biotinidase, which hydrolyzes the biotin moiety resulting in the formation of the Biotinidase Activity Assay Product.[3] A schematic of this process is shown in Figure 1, below. Using the Biotinidase Activity MaxSpec® Assay Kit, biotinidase activity may be quantified directly by LC-MS in combination with the Biotinidase Activity MaxSpec® Assay Internal Standard.

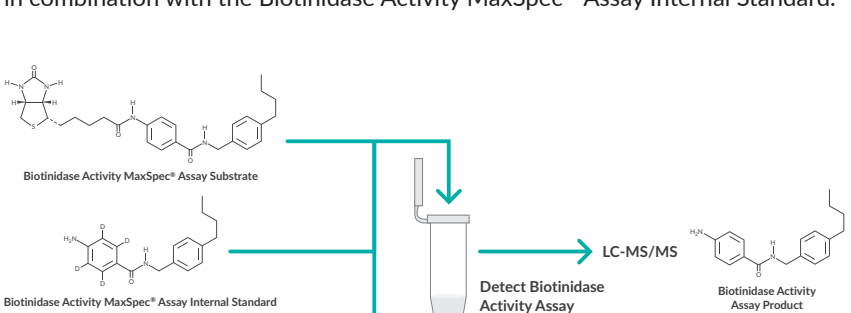

Figure 1. Biotinidase Activity MaxSpec® Assay Kit Workflow

ASSAY PROTOCOL

The procedure for quantifying biotinidase enzyme activity in dried blood spot punches has been described in detail previously.[3] This procedure will prepare enough Assay Mix for 100 tests.

Biotinidase Assay Mix Preparation

NOTE: Prepare the Assay Mix immediately prior to use.

NOTE: The Biotinidase 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.

1. Prepare the Biotinidase Activity Assay Buffer (Item No. 27543) by adding 5 ml of deionized water directly into the provided container and adjusting the pH to 7.5 with acetic acid.
2. Add 240 µl of Biotinidase Activity MaxSpec® Assay Substrate (5 mM) (Item No. 27541) and 240 µl of Biotinidase Activity MaxSpec® Assay Internal Standard (25 µM) (Item No. 27542) to a clean 5 ml vial.
3. Evaporate the solvent from the Biotinidase Activity MaxSpec® Assay Substrate and Biotinidase Activity MaxSpec® Assay Internal Standard mixture under a gentle stream of nitrogen and then add 3 ml of the diluted Biotinidase Activity Assay Buffer and vortex thoroughly.

NOTE: Batch-specific concentrations should be used for all final calculations.

Performing the Assay

1. Add a 3 mm dried blood spot (DBS) punch and 10 µl of diluted Biotinidase Activity Assay Buffer to one well of a deep-well 96-well plate.
2. Add 30 µl of Biotinidase Assay Mix. Repeat steps 1 and 2 for all samples.
3. Seal the plate with a polytetrafluoroethylene (PTFE) mat and incubate at 37°C on an orbital shaker at 250 rpm for 3 hours.

LC-MS/MS Assay Readout

1. Quench the reaction by adding 100 µl of a 1:1 solution of ethyl acetate:methanol.
2. Add an additional 400 µl of ethyl acetate and 200 µl of 0.5 M aqueous sodium chloride.
3. Mix by aspiration with a pipettor 10 times and then centrifuge at 3,000 x g for 5 minutes at room temperature.
4. Transfer 200 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.
5. Add 100 µl of a 65:35 solution of methanol:water with 5 mM ammonium acetate and mix by aspiration with a pipettor 10 times.
6. Seal the plate with foil and place in an autosampler at 8°C for LC-MS/MS analysis.

LC-MS/MS Quantification Method

LC-MS/MS Analysis

After sample preparation, the Biotinidase Activity Assay Product and the Biotinidase Activity MaxSpec® Assay Internal Standard were selectively monitored on a Waters Xevo TQD Triple Quadrupole Mass Spectrometer, which was integrated with a Waters ACQUITY UPLC system. Detailed LC-MS/MS method is described below, which can be easily adapted to a variety of LC-MS/MS systems.

HPLC Settings

A Waters XBridge BEH C8 XP column (2.1 x 50 mm, 2.5 µm) fitted with a 2.7 µm guard column was used for HPLC separation. The column was operated at 40°C and the flow rate was 0.45 ml/min.

Mobile Phase Solvent A: A 50:50 solution of water:methanol with 5 mM ammonium acetate

Mobile Phase Solvent B: A 50:50 solution of acetonitrile:methanol with 5 mM ammonium acetate

HPLC Gradient Program

Time (minutes)	%A	%B
0	75	25
0.80	75	25
0.85	0	100
1.75	0	100
1.85	75	25
2.30	75	25

Mass Spectrometry Settings

Parameter	Value
Capillary Voltage	3.5 kV
Desolvation Temperature	500°C
Source Temperature	150°C
Desolvation Gas Flow	1,000 L/hr
Cone Gas Flow	30 L/hr

Multiple Reaction Monitoring Parameters*

Compound	Parent (*m/z*)	Daughter (*m/z*)	Dwell (ms)	Cone (V)	Collision (V)
Biotinidase Activity Assay Product	283.3	91	25	26	32
Biotinidase Activity MaxSpec® Internal Standard	287.2	91	25	26	32

*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}) = [(biotinidase product area/biotinidase internal standard area) x µmoles of internal standard in assay well] / [(incubation time (h) * DBS volume (L))]

OR

A_e (µM/hour) = [(biotinidase product area/ biotinidase internal standard area) * ($6e^{-3}$ µmol)] / [(3 hours * $1.6e^{-6}$ L)]

The estimated volume in a 3 mm DBS punch is 1.6 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; 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. Zempleni, J., Hassan, Y.I., and Wigeratne, S.S.K. *Expert. Rev. Endocrinol. Metab.* **3(6)**, 715-724 (2008).
2. Wolf, B. *GeneReviews® [Internet]* (2000).
3. Hong, X., Kumar, A.B., Scott, C.R., *et al. Mol. Genet. Metab.* **124(2)**, 101-108 (2018).

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.