



Cambridge Isotope Laboratories, Inc.  
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MS/MS STANDARDS

# MS/MS Screening Mixtures and Standards



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## Amino Acid Reference Standard Mixtures (NSK-A and NSK-A1)

These sets contain 10 vials of a dry mixture of 12 stable isotope-labeled amino acids. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of solvent (e.g., 1:1 purified water:methanol).
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approaches for Working Stock

To prepare working stock solutions:

- Dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.
- If NSK-B (Acylcarnitine Standard Mix B) was purchased, mix 1 mL (or an aliquot of the reconstituted vial contents) of concentrated standards from NSK-A with 1 mL (or an aliquot) of the concentrated standards from mix B.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)
L-Alanine (Ala)	2,3,3,3-D <sub>4</sub> , 98%	93.12	500
L-Arginine-HCl (Arg)	5- <sup>13</sup> C, 99%; 4,4,5,5-D <sub>4</sub> , 95%	215.68	500
L-Aspartic acid (Asp)	2,3,3-D <sub>3</sub> , 98%	136.12	500
L-Citrulline (Cit)	5,5-D <sub>2</sub> , 98%	177.20	500
DL-Glutamic acid (Glu)	2,4,4-D <sub>3</sub> , 98%	150.15	500
Glycine (Gly)	2- <sup>13</sup> C, 99%; <sup>15</sup> N, 98%	77.05	2500
L-Leucine (Leu)	5,5,5-D <sub>3</sub> , 99%	134.19	500
L-Methionine (Met)	methyl-D <sub>3</sub> , 98%	152.23	500
L-Ornithine-HCl (Orn)*	5,5-D <sub>2</sub> , 98%	170.63	500
L-Phenylalanine (Phe)	ring- <sup>13</sup> C <sub>6</sub> , 99%	171.15	500
L-Tyrosine (Tyr)	ring- <sup>13</sup> C <sub>6</sub> , 99%	187.14	500
L-Valine (Val)	D <sub>8</sub> , 98%	125.20	500

\*NSK-A1 contains Orn 3,3,4,4,5,5,-D<sub>6</sub>, 98% (MW 174.66 Da). The remaining components are equivalent to NSK-A.

**Note:** A complementary mix of these unlabeled amino acid reference standards (NSK-A-US) and/or a combined set with NSK-B (i.e., NSK-AB) is also available.

### Usage Specifications

Criteria	Recommendation
Use	960 samples/vial
<b>Before reconstitution:</b>	
Storage	≤25°C; protect from light
Recommended retest	4 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 2-8°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## 3-Plex Amino Acid Reference Standard Mixtures (NSK-AA3)

This set contains 10 vials of a dry mixture of three stable isotope-labeled amino acids. Accurate and complete reconstitution of the vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below.

### Example Reconstitution Approach for Concentrated Stock

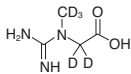
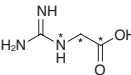
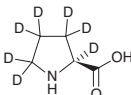
- Solubilize the dried-down mix in 1 mL of solvent (e.g., 1:1 purified water:acetonitrile, water).
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) of the concentrated amino acid standard with pure solvent.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)	Structure
Creatine (Cre)	N-Methyl-D <sub>3</sub> ; glycine-2,2-D <sub>2</sub> , 99%	154.18	500	
Guanidinoacetic acid (GAA)	1,2- <sup>13</sup> C <sub>2</sub> , 97%; 3- <sup>15</sup> N, 97% (CP 97%)	120.09	50	
L-Proline (Pro)	D <sub>7</sub> , 97%	122.17	500	

**Note:** The concentrations are also available at 10x (NSK-AA3-10X).

### Usage Specifications

Criteria	Recommendation
<b>Before reconstitution:</b>	
Storage	2-8°C; protect from light
Recommended retest	3 years from date of manufacture
<b>After reconstitution:*</b>	
Storage	-20°C
Recommended retest	3 months

\*Represents minimum stability period when AA3 mix is reconstituted with purified water:acetonitrile (1:1).

## Branched-Chain Amino Acid Reference Standard Mixtures (NSK-BCAA)

This dried-down mix comprises four stable isotope-labeled amino acids. Accurate and complete reconstitution of the vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of solvent (e.g., 0.1 M HCl, water).
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) of the concentrated amino acid standard with pure solvent.

### Composition

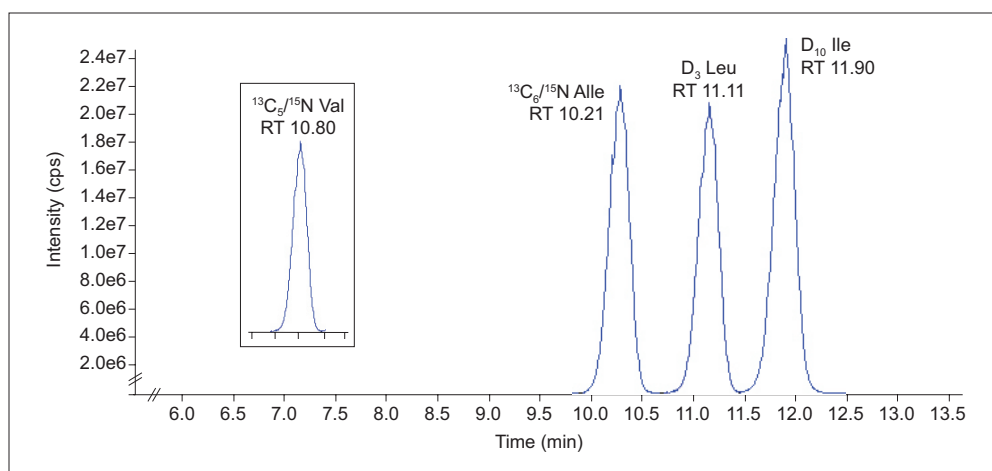
Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)
L-Allo-isoleucine (Allo)	$^{13}\text{C}_6$ , 97%; $^{15}\text{N}$ , 97%	138.2	400
L-Isoleucine (Ile)	$\text{D}_{10}$ , 98%	141.2	400
L-Leucine (Leu)	5,5,5- $\text{D}_3$ , 99%	134.2	400
L-Valine (Val)	$^{13}\text{C}_5$ , 99%; $^{15}\text{N}$ , 99%	123.1	400

**Note:** A mix of unlabeled BCAA standards (NSK-BCAA-US) is also available.

### Usage Specifications

Criteria	Recommendation
<b>Before reconstitution:</b>	
Storage	≤25°C; protect from light and moisture
Recommended retest	5 years from date of manufacture
<b>After reconstitution:*</b>	
Storage	4°C
Recommended retest	5 weeks

\*Represents minimum stability period when the BCAA mix is reconstituted with 100% water.



**Figure.** Representative XICs of the BCAA mix metabolites measured by RPLC-MRM/MS (ESI+, 5500+ QqQ). Separation of the isobaric metabolites is shown in the main plot, with Val in the inset. Displayed are the  $[\text{M}+\text{H}]^+$  ions (see Table on page 16 for precursor and product ion values).

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Carnitine and Acylcarnitine Reference Standard Mixtures (NSK-B)

This set contains 10 vials of a dry mixture of eight stable isotope-labeled free carnitine and acylcarnitines. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of highly pure methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approaches for Working Stock

To prepare working stock solutions:

- Dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.
- If NSK-A (Amino Acid Standard Mix A) was purchased, mix 1 mL (or an aliquot) of the reconstituted vial contents of concentrated standards from NSK-B with 1 mL (or an aliquot) of the concentrated standards from mix A.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)
L-Carnitine (C0)	trimethyl-D <sub>9</sub> , 98%	170.25	152
O-Acetyl-L-carnitine-HCl (C2)	N-methyl-D <sub>3</sub> , 98%	242.72	38
O-Propionyl-L-carnitine-HCl (C3)	N-methyl-D <sub>3</sub> , 98%	256.74	7.6
O-Butyryl-L-carnitine-HCl (C4)	N-methyl-D <sub>3</sub> , 98%	270.77	7.6
O-Isovaleryl-L-carnitine-HCl (C5)	N,N,N-trimethyl-D <sub>9</sub> , 98%	290.83	7.6
O-Octanoyl-L-carnitine-HCl (C8)	N-methyl-D <sub>3</sub> , 98%	326.87	7.6
O-Myristoyl-L-carnitine-HCl (C14)	N,N,N-trimethyl-D <sub>9</sub> , 98%	417.07	7.6
O-Palmitoyl-L-carnitine-HCl (C16)	N-methyl-D <sub>3</sub> , 98%	439.09	15.2

**Note:** A complementary mix of these unlabeled carnitine/acylcarnitine standards (NSK-B-US) and a combined set with NSK-A (i.e., NSK-AB) or NSK-B-G1 (i.e., NSK-BBG1) is also available.

### Usage Specifications

Criteria	Recommendation
Use	960 samples/vial
<b>Before reconstitution:</b>	
Storage	-20°C; protect from light
Recommended retest	1.5 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 2-8°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

### Example References

Kilroe, S.P.; Von Ruff, Z.D.; Arentson-Lantz, E.J.; et al. **2025**. Human skeletal muscle disuse atrophy has profound and negative effects on the muscle metabolome and lipidome. *Am J Physiol Endocrinol Metab*, 328(6), E962-E978.

The relationship between islet β-cell function and metabolomics in overweight patients with Type 2 diabetes. *Biosci Rep*, 43(2), BSR20221430-BSR20221444.

Schupper, A.; Almashanu, S.; Coster, D.; et al. **2021**. Metabolic biomarkers of small and large for gestational age newborns. *Early Hum Dev*, 160, 105422-105429.

Staretz-Chacham, O.; Daas, S.; Ulanovsky, I.; et al. **2021**. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inher Metab Dis*, 44(3), 606-617.

Brennenstuhl, H.; Kohlmüller, D.; Gramer, G.; et al. **2020**. High throughput newborn screening for aromatic L-amino-acid decarboxylase deficiency by analysis of concentrations of 3-O-methyldopa from dried blood spots. *J Inher Metab Dis*, 43(3), 602-610.

Bai, Q.; Peng, B.; Wu, X.; et al. **2018**. Metabolomic study for essential hypertension patients based on dried blood spot mass spectrometry approach. *IUBMB Life*, 70(8), 777-785.

Céspedes, N.; Valencia, A.; Echeverry, C.A.; et al. **2017**. Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colomb Med (Cali)*, 48(3), 113-119.

Haynes, C.A.; De Jesús, V.R. **2016**. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Biochem*, 49(1-2), 161-165.

Wang, Q.; Sun, T.; Cao, Y.; et al. **2016**. A dried blood spot mass spectrometry metabolomic approach for rapid breast cancer detection. *Onco Targets Ther*, 9, 1389-1398.

Huang, T.; Cao, Y.; Zeng, J.; et al. **2016**. Tandem mass spectrometry-based newborn screening strategy could be used to facilitate rapid and sensitive lung cancer diagnosis. *Onco Targets Ther*, 9, 2479-2487.

George, R.S.; Moat, S.J. **2016**. Effect of dried blood spot quality on newborn screening analyte concentrations and recommendations for minimum acceptance criteria for sample analysis. *Clin Chem*, 62(3), 466-475.

**Note:** These references utilize NSK-A and NSK-B.

### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. (Thermo Scientific technical note #73398).

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Supplemental Acylcarnitine Reference Standard Mixtures (NSK-B-G1)

This set contains 10 vials of a dry mixture of five stable isotope-labeled acylcarnitines. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of highly pure methanol.
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approaches for Working Stock

To prepare working stock solutions:

- Dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.
- Mix 1 mL (or an aliquot) of the reconstituted vial contents of concentrated standards from NSK-A with 1 mL (or an aliquot) of the concentrated standards from NSK-B and 1 mL (or an aliquot) of the concentrated standards from NSK-B-G1.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)
O-Glutaryl-L-carnitine-ClO <sub>4</sub> (C5-DC)	N-methyl-D <sub>3</sub> , 98% (CP 97%)	378.78	15.2
3-Hydroxyisovaleryl-L-carnitine-ClO <sub>4</sub> (C5-OH)	N-methyl-D <sub>3</sub> , 98%	364.79	7.6
O-Dodecanoyl-L-carnitine-HCl (C12)	N,N,N-trimethyl-D <sub>9</sub> , 98%	389.02	7.6
O-3-DL-Hydroxypalmitoyl-L-carnitine-ClO <sub>4</sub> (C16-OH)	N-methyl-D <sub>3</sub> , 98%	519.08	15.2
O-Octadecanoyl-L-carnitine-HCl (C18)	N-methyl-D <sub>3</sub> , 98%	467.15	15.2

### Usage Specifications

Criteria	Recommendation
Use	960 samples/vial
<b>Before reconstitution:</b>	
Storage	2-8°C; protect from light
Recommended retest	2 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 2-8°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

**Note:** A complementary mix of these unlabeled acylcarnitine standards (NSK-B-G1-US) and a combined set with NSK-B (NSK-BBG1) or NSK-B-US (NSK-BBG1-US) is also available.

### Example References

Schupper, A.; Almashanu, S.; Coster, D.; et al. **2021**. Metabolic biomarkers of small and large for gestational age newborns. *Early Hum Dev*, 160, 105422.

Staretz-Chacham, O.; Daas, S.; Ulanovsky, I.; et al. **2021**. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inherit Metab Dis*, 44(3), 606-617.

Céspedes, N.; Valencia, A.; Echeverry, C.A.; et al. **2017**. Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colomb Med (Cali)*, 48(3), 113-119.

Simcox, J.; Geoghegan, G.; Maschek, J.A.; et al. **2017**. Global analysis of plasma lipids identifies liver-derived acylcarnitines as a fuel source for brown fat thermogenesis. *Cell Metab*, 26(3), 509-522.

Haynes, C.A.; De Jesús, V.R. **2016**. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Biochem*, 49(1-2), 161-165.

George, R.S.; Moat, S.J. **2016**. Effect of dried blood spot quality on newborn screening analyte concentrations and recommendations for minimum acceptance criteria for sample analysis. *Clin Chem*, 62(3), 466-475.

### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. (Thermo Scientific technical note #73398).

## Lysophosphatidylcholine Reference Standard Mixtures (NSK-LPC)

This dried-down mix comprises four lysophosphatidylcholine (LPC or Lyso-PC) standards. Accurate and complete reconstitution of the vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of solvent (e.g., 95%:5% v/v methanol:water).
- Sonicate the reconstituted vial for 3 minutes then auto-vortex for 10 seconds or until complete dissolution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)	Structure
Lysophosphatidylcholine 20:0 (LysoPC C20:0)	eicosanoyl-12,12,13,13-D <sub>4</sub> , 98%	555.77	5.5	
Lysophosphatidylcholine 22:0 (LysoPC C22:0)	docosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	585.75	5.5	
Lysophosphatidylcholine 24:0 (LysoPC C24:0)	tetracosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	613.81	5.5	
Lysophosphatidylcholine 26:0 (LysoPC C26:0)	hexacosanoyl-1,2,3,4,5,6- <sup>13</sup> C <sub>6</sub> , 99%	641.85	5.5	

**Note:** A mix of unlabeled LPC standards (NSK-LPC-US) is also available.

### Usage Specifications

Criteria	Recommendation
Use	~765 samples/vial
<b>Before reconstitution:</b>	
Storage	-20°C; protect from light
Recommended retest	3 years from date of manufacture
<b>After reconstitution:</b>	
Storage	5±3°C or -20±5°C
Recommended retest	6 weeks

Chemical purity (CP) is 98% or greater, unless otherwise specified.



## Steroid Reference Standard Mixtures (NSK-S and NSK-S-EXP)

The sets contain 10 vials of dried-down stable isotope-labeled steroids. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Vials of the individual mixes and compounds are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of solvent (e.g., methanol).
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted according to the following instructions, or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.

### Steroid Mix S (NSK-S)

#### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (nM)
4-Androstene-3,17-dione (A4)	2,2,4,6,6,16,16-D <sub>7</sub> , 97%	293.45	20
Cortisol (F)	9,11,12,12-D <sub>4</sub> , 98%	366.49	100
11-Deoxycortisol (11-S)	2,2,4,6,6-D <sub>5</sub> , 98%	351.49	20
21-Deoxycortisol (21-S)	2,2,4,6,6,21,21-D <sub>8</sub> , 97%	354.51	20
17 $\alpha$ -Hydroxyprogesterone (17-OHP)	2,2,4,6,6,21,21-D <sub>8</sub> , 98%	338.51	20

**Note:** The concentrations are also available at 40x (NSK-S-40X) in a single vial.

#### Usage Specifications

Criteria	Recommendation
Use	48 samples/vial
<b>Before reconstitution:</b>	
Storage	2-8°C; protect from light
Recommended retest	5 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 2-8°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

#### Example References

Lai, F.; Srinivasan, S.; Wiley, V. **2020**. Evaluation of a two-tier screening pathway for congenital adrenal hyperplasia in the New South Wales Newborn Screening Programme. *Int J Neonatal Screen*, 6(3), 63-74.

Gervasoni, J.; Schiattarella, A.; Primiano, A.; et al. **2016**. Simultaneous quantification of 17-hydroxyprogesterone, androstenedione, testosterone and cortisol in human serum by LC-MS/MS using TurboFlow online sample extraction. *Clin Biochem*, 49(13-14), 998-1003.

Hicks, R.A.; Yee, J.K.; Mao, C.S.; et al. **2014**. Precursor-to-product ratios reflect biochemical phenotype in congenital adrenal hyperplasia. *Metabolomics*, 10(1), 123-131.

Dhillon, K.; Ho, T.; Rich, P.; et al. **2011**. An automated method on analysis of blood steroids using liquid chromatography tandem mass spectrometry: application to population screening for congenital adrenal hyperplasia in newborns. *Clin Chem Acta*, 412(23-24), 2076-2084.

### Expanded Steroid Mix S (NSK-S-EXP)

#### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. ( $\mu$ M)
Aldosterone (A)	D <sub>7</sub> , 98%	367.49	0.52
4-Androstene-3,17-dione (A4)	2,2,4,6,6-D <sub>5</sub> , 98%	291.44	0.12
Corticosterone (B)	2,2,4,6,6,17 $\alpha$ ,21,21-D <sub>8</sub> , 97%	354.51	1.58
Cortisol (F)	9,12,12-D <sub>3</sub> , 98%	365.48	2.57
Dehydroepiandrosterone sulfate-sodium salt·2H <sub>2</sub> O (DHEAS)	2,2,3,4,4,6-D <sub>6</sub> , 95%	432.54	21.69
11-Deoxycortisol (11-S)	2,2,4,6,6-D <sub>5</sub> , 98% (CP 97%)	351.49	0.54
17 $\alpha$ -Hydroxyprogesterone (17-OHP)	2,2,4,6,6,21,21-D <sub>8</sub> , 98%	338.51	0.27
Progesterone (P)	2,2,4,6,6,17 $\alpha$ ,21,21-D <sub>9</sub> , 98%	323.52	0.14
Testosterone (T)	2,2,4,6,6-D <sub>5</sub> , 98%	293.46	0.12

#### Usage Specifications

Criteria	Recommendation
<b>Before reconstitution:</b>	
Storage	2-8°C; protect from light
Recommended retest	1 year from date of manufacture

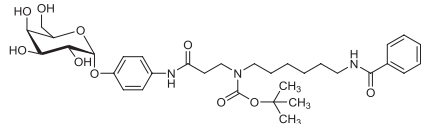
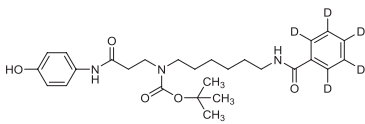
Chemical purity (CP) is 98% or greater, unless otherwise specified.



## Additional Standard Mixtures

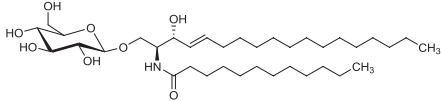
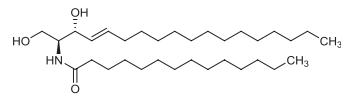
### $\alpha$ -Galactosidase Substrate and Internal Standard Mix (NSK-FA)

Each vial contains the following compounds at a substrate:internal standard (S:IS) molar ratio of 500:1.

Substrate	Internal Standard
6-Benzoylamino-hexyl-[2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenylcarbamoyl]-ethyl]-carbamic acid <i>tert</i> -butyl ester	6-D <sub>5</sub> -Benzoylamino-hexyl-[2-(4-hydroxy-phenyl-carbamoyl)-ethyl]-carbamic acid <i>tert</i> -butyl ester
C <sub>33</sub> H <sub>47</sub> N <sub>3</sub> O <sub>10</sub> MW: 645.7 Da	C <sub>27</sub> H <sub>32</sub> N <sub>3</sub> O <sub>5</sub> D <sub>5</sub> MW: 488.5 Da
	

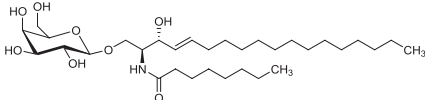
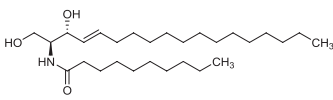
### Glucocerebrosidase Substrate and Internal Standard Mix (NSK-GA)

Each vial contains the following compounds at a substrate:internal standard (S:IS) molar ratio of 50:1.

Substrate	Internal Standard
D-Glucosyl- $\beta$ 1-1'- <i>N</i> -dodecanoyl-D- <i>erythro</i> -sphingosine (C12-glucocerebroside)	<i>N</i> -Myristoyl-D- <i>erythro</i> -sphingosine (C14-ceramide)
C <sub>36</sub> H <sub>69</sub> NO <sub>8</sub> MW: 643.9 Da	C <sub>32</sub> H <sub>63</sub> NO <sub>3</sub> MW: 509.8 Da
	

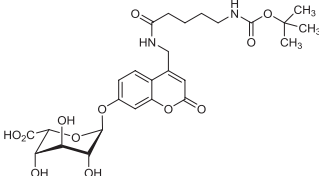
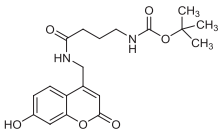
### Galactocerebrosidase Substrate and Internal Standard Mix (NSK-KR)

Each vial contains the following compounds at a substrate:internal standard (S:IS) molar ratio of 150:1.

Substrate	Internal Standard
D-Galactosyl- $\beta$ 1-1'-octanoyl-D- <i>erythro</i> -sphingosine (C8-galactosylceramide)	<i>N</i> -Decanoyl-D- <i>erythro</i> -sphingosine (C10-ceramide)
C <sub>32</sub> H <sub>61</sub> NO <sub>8</sub> MW: 587.8 Da	C <sub>28</sub> H <sub>55</sub> NO <sub>3</sub> MW: 453.7 Da
	

### $\alpha$ -L-Iduronidase Substrate and Internal Standard Mix (NSK-MP)

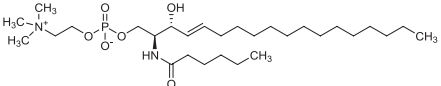
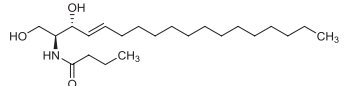
Each vial contains the following compounds at a substrate:internal standard (S:IS) molar ratio of 150:1.

Substrate	Internal Standard
7-(1-Iduronic acid)-oxycoumarin-4-methylamine-(5'- <i>N</i> -boc-aminopentanoyl)-amide	7-Hydroxycoumarin-4-methylamine-(4'- <i>N</i> -boc-aminobutanoyl)-amide
C <sub>26</sub> H <sub>34</sub> N <sub>2</sub> O <sub>12</sub> MW: 566.6 Da	C <sub>19</sub> H <sub>24</sub> N <sub>2</sub> O <sub>6</sub> MW: 376.4 Da
	

## Additional Standard Mixtures *(continued)*

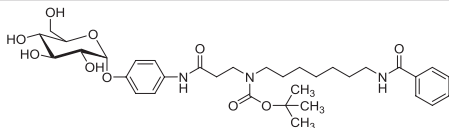
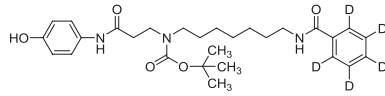
### Acid Sphingomyelinase Substrate and Internal Standard Mix (NSK-NI)

Each vial contains the following compounds at a substrate:internal standard (S:IS) molar ratio of 50:1.

Substrate	Internal Standard
<i>N</i> -Hexanoyl-D-erythro-sphingosylphosphorylcholine (C6-sphingomyelin)	<i>N</i> -Butyroyl-D-erythro-sphingosine (C4-ceramide)
$C_{29}H_{59}N_2O_6P$ MW: 562.8 Da	$C_{22}H_{43}NO_3$ MW: 369.6 Da
	

### Acid $\alpha$ -Glucosidase Substrate and Internal Standard Mix (NSK-PO)

Each vial contains the following compounds at a substrate:internal standard (S:IS) molar ratio of 100:1.

Substrate	Internal Standard
7-Benzoylamino-heptyl-[2-[4-(3,4,5-trihydroxy-6-hydroxymethyl-tetrahydropyran-2-yloxy)-phenylcarbamoyl]-ethyl]-carbamic acid <i>tert</i> -butyl ester	7-D <sub>5</sub> -Benzoylamino-heptyl-[2-(4-hydroxy-phenyl-carbamoyl)-ethyl]-carbamic acid <i>tert</i> -butyl ester
$C_{34}H_{49}N_3O_{10}$ MW: 659.8 Da	$C_{28}H_{34}N_3O_5D_5$ MW: 502.7 Da
	

### Usage Specifications

Criteria	Recommendation
Use	~600 samples/vial

Before reconstitution:		After reconstitution:	
Storage	-20°C; protect from light	Storage	5±3°C or -20±5°C
Recommended retest	2 years from date of manufacture	Recommended retest	4 weeks

### Example References

Ribas, G.; De Mari, J.F.; Civallero, G.; et al. **2017**. Validation of a multiplex tandem mass spectrometry method for the detection of selected lysosomal storage diseases in dried blood spots. *JIMES*, 5, 1-7.

Tortorelli, S.; Turgeon, C.T.; Gavrilov, D.K.; et al. **2016**. Simultaneous testing for 6 lysosomal storage disorders and x-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. *Clin Chem*, 62(9), 1248-1254.

Cho, S.E.; Kwak, J.R.; Lee, H.; et al. **2016**. Triplex tandem mass spectrometry assays for the screening of 3 lysosomal storage disorders in a Korean population. *Clin Chim Acta*, 454, 20-27.

## Succinylacetone Reference Standard (NSK-T)

This set contains 10 vials of dried-down stable isotope-labeled succinylacetone. Accurate and complete reconstitution of one vial's contents in 1 mL of high-purity solvent will produce the concentrations listed in the table below. Individual vials are also available.

### Example Reconstitution Approach for Concentrated Stock

- Solubilize the dried-down mix in 1 mL of solvent (e.g., methanol).
- Vortex manually for 1 minute then auto-vortex for 30 minutes or until complete reconstitution is achieved.

Aliquots of the concentrated stock can then be processed, diluted, and/or stored as indicated below.

### Example Dilution Approach for Working Stock

To prepare working stock solutions, dilute 1 mL (or an aliquot) of the reconstituted vial contents (per instructions above) with pure solvent.

### Composition

Standard (Abbreviation)	Label and Enrichment	MW (Da)	Conc. (μM)
Succinylacetone (SUAC)	3,4,5,6,7- <sup>13</sup> C <sub>5</sub> , 99%	163.12	1000

**Note:** An unlabeled solution of the SUAC standard (NSK-T-US, 0.1 mg/mL) is also available, as is a dried-down <sup>13</sup>C<sub>5</sub>-labeled SUAC standard (CLM-6755, 10 mg).

### Usage Specifications

Criteria	Recommendation
Use	9,600 samples/vial
<b>Before reconstitution:</b>	
Storage	≤25°C; protect from light and moisture
Recommended retest	5 years from date of manufacture
<b>After reconstitution:</b>	
Storage	Store in a tightly sealed vial at 2-8°C. To maintain the integrity of the solution, store the sealed vials in a second sealed container.
Recommended retest	4 weeks

### Example References

Fuenzalida, K.; Leal-Witt, M.J.; Guerrero, P.; et al. **2021**. NTBC treatment monitoring in Chilean patients with tyrosinemia type 1 and its association with biochemical parameters and liver biomarkers. *J Clin Med*, 10(24), 5832-5845.

Schupper, A.; Almashanu, S.; Coster, D.; et al. **2021**. Metabolic biomarkers of small and large for gestational age newborns. *Early Hum Dev*, 160, 105422-105429.

Staretz-Chacham, O.; Daas, S.; Ulanovsky, I.; et al. **2021**. The role of orotic acid measurement in routine newborn screening for urea cycle disorders. *J Inher Metab Dis*, 44(3), 606-617.

Brennenstuhl, H.; Kohlmüller, D.; Gramer, G.; et al. **2020**. High throughput newborn screening for aromatic L-amino-acid decarboxylase deficiency by analysis of concentrations of 3-O-methyldopa from dried blood spots. *J Inher Metab Dis*, 43(3), 602-610.

Jack, R.M.; Scott, C.R. **2019**. Validation of a therapeutic range for nitisinone in patients treated for tyrosinemia type 1 based on reduction of succinylacetone excretion. *JIMD Reports*, 46(1), 75-78.

Céspedes, N.; Valencia, A.; Echeverry, C.A.; et al. **2017**. Reference values of amino acids, acylcarnitines and succinylacetone by tandem mass spectrometry for use in newborn screening in southwest Colombia. *Colomb Med (Cali)*, 48(3), 113-119.

de Sain-van der Velden, M.G.M.; van der Ham, M.; Gerrits, J.; et al. **2017**. Quantification of metabolites in dried blood spots by direct infusion high resolution mass spectrometry. *Anal Chim Acta*, 979, 45-50.

Haynes, C.A.; De Jesús, V.R. **2016**. Simultaneous quantitation of hexacosanoyl lysophosphatidylcholine, amino acids, acylcarnitines, and succinylacetone during FIA-ESI-MS/MS analysis of dried blood spot extracts for newborn screening. *Clin Biochem*, 49(1-2), 161-165.

Pankowicz, F.P.; Barzi, M.; Legras, X.; et al. **2016**. Reprogramming metabolic pathways *in vivo* with CRISPR/Cas9 genome editing to treat hereditary tyrosinaemia. *Nat Commun*, 7, 12642-12647.

### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. (Thermo Scientific technical note #73398).

## Other Standards

Catalog No.	Abbreviated Standard	Abbreviation	Label and Enrichment	Unit Size
CLM-3777	<i>N</i> -Acetylglycine	AG	2- <sup>13</sup> C, 99%	Please inquire
CLM-3678	Adenosine	Ado	ribose- <sup>13</sup> C <sub>5</sub> , 98% (CP 97%)	0.05 g, 0.1 g
CLM-8906	5-Adenosyl-L-homocysteine	SAH	adenosine- <sup>13</sup> C <sub>10</sub> , 98% (CP 95%)	0.1 mg
CNLM-3946	β-Alanine	BALA	<sup>13</sup> C <sub>3</sub> , 98%; <sup>15</sup> N, 96%	0.25 g
DLM-10008	5-Androstan-3α-ol-17-one (etiocholanolone)	etio	2,2,3,4,4-D <sub>5</sub> , 98%	1 mg
CNLM-9007	L-Argininosuccinic acid, barium salt·2H <sub>2</sub> O	ASA	arginine- <sup>13</sup> C <sub>6</sub> , 99%; <sup>15</sup> N <sub>4</sub> , 99% (CP 90%)	0.1 mg, 0.5 mg
DLM-11049	L-Carnitine·ClO <sub>4</sub> , <i>O</i> -malonyl	C3-DC	<i>N</i> -methyl-D <sub>3</sub> , 98% (CP 95%)	0.1 mg
DLM-11877	L-Carnitine·HCl, <i>O</i> -methylmalonyl	C4-DC	<i>N</i> -methyl-D <sub>3</sub> , 98% (CP 90%)	Please inquire
DLM-11769	L-Carnitine·HCl, <i>O</i> -3-hydroxybutyryl	C4-OH	<i>N</i> -methyl-D <sub>3</sub> , 98% (CP 95%)	0.1 mg
DLM-12325	L-Carnitine·ClO <sub>4</sub> , tiglyl (C5:1)	C5:1	<i>N,N,N</i> -trimethyl-D <sub>3</sub> , 98% (CP 90%)	5 mg
DLM-9276	<i>O</i> -Hexanoyl-L-carnitine·HCl	C6	<i>N</i> -methyl-D <sub>3</sub> , 98%	0.1 mg
DLM-9067	<i>O</i> -Decanoyl-L-carnitine·HCl	C10	<i>N</i> -methyl-D <sub>3</sub> , 98%	0.1 mg
DLM-8746	L-Carnitine·HCl, <i>O</i> -dec-2-enoyl	C10:1	<i>N,N,N</i> -trimethyl-D <sub>3</sub> , 98% (95% E)	1 mg
DLM-12326	L-Carnitine·ClO <sub>4</sub> , tetradec-5- <i>cis</i> -enoyl	C14:1	<i>N,N,N</i> -trimethyl-D <sub>3</sub> , 98% (CP 90%)	5 mg
DLM-11715	L-Carnitine·HCl, <i>O</i> -hydroxyoctadecanoyl	C18-OH	<i>N</i> -methyl-D <sub>3</sub> , 95% (CP 95%)	0.1 mg
DLM-6718	<i>O</i> -Hexacosanoyl-L-carnitine·HCl	C26	<i>N</i> -methyl-D <sub>3</sub> , 98% (CP 95%)	Please inquire
DLM-7347	Corticosterone	B	2,2,4,6,6,17α,21,21-D <sub>8</sub> , 97%	0.01 g
DLM-9976	Cortisone	E	2,2,4,6,6,9,12,12-D <sub>8</sub> , 98%	1 mg, 5 mg
CLM-7933	Creatine	Cre	guanidino- <sup>13</sup> C, 99%	0.1 g
DLM-1302	Creatine	Cre	methyl-D <sub>3</sub> , 98% (CP 97%)	0.25 g
DLM-3653	Creatinine	Crn	<i>N</i> -methyl-D <sub>3</sub> , 98%	0.1 g
CLM-10549	Dehydroepiandrosterone	DHEA	2,3,4- <sup>13</sup> C <sub>3</sub> , 99%	1 mg
DLM-8049	Dehydroepiandrosterone	DHEA	2,2,3,4,4,6-D <sub>6</sub> , 98% (CP 97%)	5 mg
CLM-10784	Dehydroepiandrosterone sulfate, sodium salt	DHEAS	2,3,4- <sup>13</sup> C <sub>3</sub> , 98%	1 mg
DLM-8337	Dehydroepiandrosterone sulfate, sodium salt·2H <sub>2</sub> O	DHEAS	2,2,3,4,4,6-D <sub>6</sub> , 95%	5 mg
CLM-4579	2'-Deoxyadenosine·H <sub>2</sub> O	dAdo	ribose- <sup>13</sup> C <sub>5</sub> , 99%	Please inquire
DLM-7687	2'-Deoxyguanosine·H <sub>2</sub> O	2dG	ribose-5,5-D <sub>2</sub> , 98%	0.05 g, 0.1 g
DLM-3023	Dihydrotestosterone	DHT	16,16,17-D <sub>3</sub> , 98%	Please inquire
CLM-7824	L-Dihydroxyphenylalanine	L-Dopa	1- <sup>13</sup> C, ring- <sup>13</sup> C <sub>6</sub> , 99%	0.05 g
DLM-2084	L-Dihydroxyphenylalanine	L-Dopa	ring-D <sub>3</sub> , 98%	1 g
CLM-803	Estradiol	E2	3,4- <sup>13</sup> C <sub>2</sub> , 99%	Please inquire
DLM-2487	Estradiol	E2	2,4,16,16-D <sub>4</sub> , 95%	5 mg
CLM-9148	Estrone	E1	2,3,4- <sup>13</sup> C <sub>3</sub> , 99%	1 mg, 5 mg
DLM-3976	Estrone	E1	2,4,16,16-D <sub>4</sub> , 97%	5 mg
DLM-6013	Ethylmalonic acid	EMA	methyl-D <sub>3</sub> , 98%	0.1 g
CLM-1570	D-Galactose	Gal	U- <sup>13</sup> C <sub>6</sub> , 99%	0.1 g
CLM-9874	D-Galactose-1-phosphate, dipotassium salt	Gal-1P	galactose- <sup>13</sup> C <sub>6</sub> , 99%	Please inquire
CLM-1822-H	L-Glutamine	Gln	<sup>13</sup> C <sub>5</sub> , 99%	0.1 g, 0.25 g, 0.5 g
DLM-1826	L-Glutamine	Gln	2,3,3,4,4-D <sub>5</sub> , 97%	0.1 g
CLM-1017	Glycine	Gly	<sup>13</sup> C <sub>2</sub> , 97%	0.5 g, 1 g, 5 g
DLM-280	Glycine	Gly	D <sub>5</sub> , 98%	5 g
CNLM-8111	<i>N</i> -(3-Methylcrotonyl)glycine	3-MCG	glycine- <sup>13</sup> C <sub>2</sub> , 98%; <sup>15</sup> N, 98%	Please inquire
DLM-9715	<i>N</i> -(3-Phenylpropionyl)glycine	3-PPG	2,2-D <sub>2</sub> , 98%	Please inquire
DLM-9998	Guanidinoacetic acid	GAA	2,2-D <sub>2</sub> , 97%	Please inquire
DLM-7689	Guanosine·H <sub>2</sub> O	Guo	ribose-5,5-D <sub>2</sub> , 98%	Please inquire
CNLM-8448	<i>N</i> -Hexanoylglycine	HG	<sup>13</sup> C <sub>2</sub> , 97%; <sup>15</sup> N, 97% (CP 95%)	Please inquire
CLM-373	Homovanillic acid	HVA	1,2- <sup>13</sup> C <sub>2</sub> , 98%	0.1 g
DLM-2738	Homovanillic acid	HVA	phenyl-D <sub>3</sub> , 2,2-D <sub>2</sub> , 96%	0.1 g
DLM-8118	3-Hydroxyglutaric acid	3-OHGA	D <sub>5</sub> , 98%	Please inquire
CLM-9936	5-Hydroxyindole-3-acetic acid	5-HIAA	3α,4,5,6,7,7α- <sup>13</sup> C <sub>6</sub> , 98%	1.2 mL
DLM-7206	17α-Hydroxypregnenolone	17-OHP5	21,21,21-D <sub>3</sub> , 97%	Please inquire

Chemical purity (CP) is 98% or greater, unless otherwise specified.

## Other Standards *(continued)*

Catalog No.	Abbreviated Standard	Abbreviation	Label and Enrichment	Unit Size
DLM-3619	DL-Homocystine	HCY	3,3,3',3',4,4,4',4'-D <sub>8</sub> , 98%	0.5 g, 1 g
NLM-4264	Inosine	Ino	<sup>15</sup> N <sub>4</sub> , 95%	0.01 g, 0.05 g
CLM-8742	L-allo-Isoleucine	alle	<sup>13</sup> C <sub>6</sub> , 97%	Please inquire
DLM-1505	L-allo-Isoleucine	alle	D <sub>10</sub> , 98%	0.1 g
CNLM-9291	N-Isovaleryl glycine	IVG	glycine- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 99%	Please inquire
CLM-2247-H	L-Lysine·2HCl	Lys	<sup>13</sup> C <sub>6</sub> , 99%	0.05 g, 0.1 g, 0.25 g, 0.5 g, 1 g
DLM-10500	Lysophosphatidylcholine 22:0	LysoPC C22:0	docosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
DLM-10497	Lysophosphatidylcholine 24:0	LysoPC C24:0	tetracosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
DLM-10501	Lysophosphatidylcholine 26:0	LysoPC C26:0	hexacosanoyl-12,12,13,13-D <sub>4</sub> , 98%	1 mg, 5 mg
DLM-205	Malonic acid	MA	D <sub>4</sub> , 98%	50 g
DLM-11341	L-3-O-Methyl-dopa·H <sub>2</sub> O	3-OMD	methoxy-D <sub>3</sub> , 98%	Please inquire
CLM-10350	2-Methylbutyric acid	2-MBA	methyl- <sup>13</sup> C, 99%	Please inquire
DLM-2312	DL-2-Methylcitric acid	MCA	methyl-D <sub>3</sub> , 98% (CP 90%)	Please inquire
CLM-9426	Methylmalonic acid	MMA	<sup>13</sup> C <sub>4</sub> , 99%	0.1 g
DLM-387	Methylmalonic acid	MMA	methyl-D <sub>3</sub> , 98%	0.25 g
CLM-4724	L-Ornithine·HCl	Orn	<sup>13</sup> C <sub>5</sub> , 99%	0.1 g
DLM-2969	L-Ornithine·HCl	Orn	3,3,4,4,5,5-D <sub>6</sub> , 98%	0.1 g, 0.25 g
NLM-1048	Orotic acid·H <sub>2</sub> O	Oro	1,3- <sup>15</sup> N <sub>2</sub> , 98%	0.25 g
DLM-7953	Progesterone	P4	2,2,4,6,6,17α,21,21-D <sub>8</sub> , 98%	0.01 g
DLM-6896	Pregnenolone	P5	17,21,21,21-D <sub>4</sub> , 98%	0.01 g
CLM-2260-H	L-Proline	Pro	<sup>13</sup> C <sub>5</sub> , 99%	0.1 g, 0.25 g, 0.5 g
CLM-7944	3-(3-Methyl-1H-pyrazol-5-yl)propanoic acid	MPP	methyl- <sup>13</sup> C, pyrazolyl- <sup>13</sup> C <sub>3</sub> , 3- <sup>13</sup> C, 99%	Please inquire
CLM-647	Propionic acid	PPA	<sup>13</sup> C <sub>3</sub> , 99%	1 g
DLM-1919	Propionic acid	PPA	D <sub>5</sub> , 98%	5 g
CNLM-9292	N-Propionyl glycine	PG	glycine- <sup>13</sup> C <sub>2</sub> , 99%; <sup>15</sup> N, 99%	Please inquire
DLM-6874	Sarcosine·HCl (N-methylglycine·HCl)	Sar	methyl-D <sub>3</sub> , 98%	0.1 g, 0.25 g
CNLM-8183	Suberyl glycine	SG	glycine- <sup>13</sup> C <sub>2</sub> , 98%; <sup>15</sup> N, 98% (CP 95%)	Please inquire
CLM-9164	Testosterone	T	2,3,4- <sup>13</sup> C <sub>3</sub> , 99%	5 mg, 10 mg
DLM-8085	Testosterone	T	2,2,4,6,6-D <sub>5</sub> , 98%	Please inquire
CLM-6725	L-Thyroxine	T4	tyrosine-ring- <sup>13</sup> C <sub>6</sub> , 99% (CP 90%)	0.1 mg
CLM-8931	L-Thyroxine	T4	ring- <sup>13</sup> C <sub>12</sub> , 99% (CP 97%)	0.1 mg
CLM-11320	L-Tyrosine	Tyr	N-acetyl (ring- <sup>13</sup> C <sub>6</sub> , 99%)	Please inquire
DLM-10758	Trisodium 2-methylcitrate, racemic mixture of diastereomers	MCA	methyl-D <sub>3</sub> , 98% (CP 90%)	5 mg, 10 mg
CLM-4290-H	L-Tryptophan	Trp	<sup>13</sup> C <sub>11</sub> , 99%	0.1 g
DLM-6903	L-Tryptophan	Trp	D <sub>8</sub> , 97%	0.25 g

**Note:** Unlabeled standards are also available. Please inquire for size and pricing or visit [isotope.com](https://isotope.com).

### Example References

Daas, S.; Salah, N.A.; Anikster, Y.; et al. **2023**. Addition of galactose-1-phosphate measurement enhances newborn screening for classical galactosemia. *J Inherit Metab Dis*, 46(2), 232-242.

Young, A.; Hendricks, J.; Foreman, D.; et al. **2020**. Development of dried blood spot quality control materials for adenosine deaminase severe combined immunodeficiency and an LC-MS/MS method for their characterization. *Clin Mass Spec*, 17, 4-11.

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Monostori, P.; Klinke, G.; Richter, S.; et al. **2017**. Simultaneous determination of 3-hydroxypropionic acid, methylmalonic acid and methylcitric acid in dried blood spots: Second-tier LC-MS/MS assay for newborn screening of propionic acidemia, methylmalonic acidemias and combined remethylation disorders. *PLoS One*, 12(9), e0184897.

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Prinsen, H.C.M.T.; Schiebergen-Bronkhorst, B.G.M.; Roeleveld, M.W.; et al. **2016**. Rapid quantification of underivatized amino acids in plasma by hydrophilic interaction liquid chromatography (HILIC) coupled with tandem mass-spectrometry. *J Inherit Metab Dis*, 39(5), 651-660.

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*Chemical purity (CP) is 98% or greater, unless otherwise specified.*

## MS/MS Parameter Examples

### Neutral Loss (NL) Scan (for NSK-A and NSK-A1 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	NL $m/z$	Precursor Ion $m/z$	NL $m/z$
Alanine ( $D_4$ )	Ala	94	46	150	102
Arginine ( $^{13}C/D_4$ )	Arg	180	105	236	161
Aspartate ( $D_3$ )	Asp	137	46	249	102
Citrulline ( $D_2$ )	Cit	178	63	234	119
Glutamate ( $D_3$ )	Glu	151	46	263	102
Glycine ( $^{13}C/^{15}N$ )	Gly	78	46	134	102
Leucine ( $D_3$ )	Leu	135	46	191	102
Methionine ( $D_3$ )	Met	153	46	209	102
Ornithine ( $D_2$ )	Orn	135	63	191	119
Ornithine ( $D_6$ )	Orn	139	63	195	119
Phenylalanine ( $^{13}C_6$ )	Phe	172	46	228	102
Tyrosine ( $^{13}C_6$ )	Tyr	188	46	244	102
Valine ( $D_8$ )	Val	126	46	182	102

**Note:** The MS/MS fragmentation mechanism of amino acids during NL scan is well established (e.g., [PMID: 14578311](#)). For example, the losses for the underivatized amino acids reflect HCOOH ( $m/z$  46), HCOOH and  $NH_3$  ( $m/z$  63), and HCOOH and  $H_2NCNHNH_2$  ( $m/z$  105).

### Precursor (Pre) Ion Scan (for NSK-B and NSK-B-G1 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	Product Ion $m/z$	Precursor Ion $m/z$	Product Ion $m/z$
Carnitine ( $D_9$ )	C0	171	85	227	85
Acetylcarnitine ( $D_3$ )	C2	207	85	263	85
Propionylcarnitine ( $D_3$ )	C3	221	85	277	85
Butyrylcarnitine ( $D_3$ )	C4	235	85	291	85
Isovalerylcarnitine ( $D_9$ )	C5	255	85	311	85
Glutaryl carnitine ( $D_3$ )	C5-DC	279	85	391	85
Hydroxyisovalerylcarnitine ( $D_3$ )	C5-OH	265	85	321	85
Octanoylcarnitine ( $D_3$ )	C8	291	85	347	85
Dodecanoylcarnitine ( $D_9$ )	C12	353	85	409	85
Myristoylcarnitine ( $D_9$ )	C14	381	85	437	85
Palmitoylcarnitine ( $D_3$ )	C16	403	85	459	85
Hydroxypalmitoylcarnitine ( $D_3$ )	C16-OH	419	85	475	85
Octadecanoylcarnitine ( $D_3$ )	C18	431	85	487	85

**Note:** The common fragment ion of  $m/z$  85 corresponds to  $^+CH_2-CH=CH-COOH$  and is consistent between nonderivatized and derivatized acylcarnitines (e.g., [PMID: 9365395](#) for background and fragmentation mechanism).

## MS/MS Parameter Examples *(continued)*

### MRM Acquisition Mode (for NSK-A, NSK-A1, and NSK-T Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	Product Ion $m/z$	Precursor Ion $m/z$	Product Ion $m/z$
Alanine ( $D_4$ )	Ala	94	48	150	48
Arginine ( $^{13}C/D_4$ )	Arg	180	75	236	75
Aspartate ( $D_3$ )	Asp	137	119	249	147
Citrulline ( $D_2$ )	Cit	178	115	234	115
Glutamate ( $D_3$ )	Glu	151	133	263	161
Glycine ( $^{13}C/^{15}N$ )	Gly	78	32	134	78
Leucine ( $D_3$ )	Leu	135	89	191	89
Methionine ( $D_3$ )	Met	153	136	209	107
Ornithine ( $D_2$ )	Orn	135	72	191	72
Ornithine ( $D_6$ )	Orn	131	68	195	68
Phenylalanine ( $^{13}C_6$ )	Phe	172	126	228	126
Tyrosine ( $^{13}C_6$ )	Tyr	188	142	244	142
Valine ( $D_8$ )	Val	126	80	182	80
Succinylacetone ( $^{13}C_5$ )	SUAC	160	114	216	142

### MRM Acquisition Mode (for NSK-B and NSK-B-G1 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Underivatized		Butyl Ester Derivatized	
		Precursor Ion $m/z$	Product Ion $m/z$	Precursor Ion $m/z$	Product Ion $m/z$
Carnitine ( $D_9$ )	C0	171	103	227	103
Acetylcarnitine ( $D_3$ )	C2	207	85	263	85
Propionylcarnitine ( $D_3$ )	C3	221	85	277	85
Butyrylcarnitine ( $D_3$ )	C4	235	85	291	85
Isovalerylcarnitine ( $D_9$ )	C5	255	85	311	85
Glutaryl carnitine ( $D_3$ )	C5-DC	279	85	335	85
Hydroxyisovalerylcarnitine ( $D_3$ )	C5-OH	265	85	321	85
Octanoylcarnitine ( $D_3$ )	C8	291	85	347	85
Dodecanoylcarnitine ( $D_9$ )	C12	353	85	409	85
Myristoylcarnitine ( $D_9$ )	C14	381	85	437	85
Palmitoylcarnitine ( $D_3$ )	C16	403	85	459	85
Hydroxypalmitoylcarnitine ( $D_3$ )	C16-OH	419	85	475	85
Octadecanoylcarnitine ( $D_3$ )	C18	431	85	487	85

#### Technical Note

Xie, X.; Kozak, M. **2020**. Simultaneous analysis of amino acids, acylcarnitines, and succinylacetone in dried blood spots for research using nonderivatized and derivatized methods. Thermo Fisher Scientific, San Jose, CA.



## MS/MS Parameter Examples *(continued)*

### MRM Acquisition Mode (for NSK-AA3 Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
Creatine ( $D_5$ )	Cre	137	95
Guanidinoacetic acid ( $^{13}C_2/^{15}N$ )	GAA	121	79
L-Proline ( $D_7$ )	Pro	123	77

### MRM Acquisition Mode (for NSK-BCAA Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
L-Allo-isoleucine ( $^{13}C_8/^{15}N$ )	Alle	139	92
L-Isoleucine ( $D_{10}$ )	Ile	142	96
L-Leucine ( $D_3$ )	Leu	135	89
L-Valine ( $^{13}C_8/^{15}N$ )	Val	124	77

### MRM Acquisition Mode (for NSK-S and NSK-S-EXP Standards)

(all  $m/z$  as  $[M+H]^+$  unless otherwise specified)

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
Aldosterone ( $D_7$ )	A	350 for $[M+H^+-H_2O]^+$	173
4-Androstene-3,17-dione ( $D_5$ )	A4	292	100
4-Androstene-3,17-dione ( $D_7$ )	A4	294	100
Corticosterone ( $D_8$ )	B	355	125
Cortisol ( $D_3$ )	F	366	121
Cortisol ( $D_4$ )	F	367	121
Dehydroepiandrosterone sulfate ( $D_6$ )	DHEAS	364	274
11-Deoxycortisol ( $D_5$ )	11-S	352	100
21-Deoxycortisol ( $D_8$ )	21-S	355	319
17 $\alpha$ -Hydroxyprogesterone ( $D_8$ )	17-OHP	339	100
Progesterone ( $D_9$ )	P	324	100
Testosterone ( $D_5$ )	T	294	100

### MRM Acquisition Mode (for NSK-LPC Standards)

(all  $m/z$  as  $[M+H]^+$ )

Compound	Abbreviation	Precursor Ion $m/z$	Product Ion $m/z$
Lysophosphatidylcholine 20:0 ( $D_4$ )	LPC 20:0	556	104
Lysophosphatidylcholine 22:0 ( $^{13}C_6$ )	LPC 22:0	586	104
Lysophosphatidylcholine 24:0 ( $^{13}C_6$ )	LPC 24:0	614	104
Lysophosphatidylcholine 26:0 ( $^{13}C_6$ )	LPC 26:0	643	104