EZ:faast™

USER'S MANUAL

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Free (Physiological) Amino Acid Analysis by LC-MS

USER'S MANUAL

For Part Numbers REF KH0-7337
REF KH0-7338

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EZ:faast® For Free Physiological Amino Acid Analysis by LC-MS

3882_L3

The following is a description of the symbols used in the EZ:faast manuals, on EZ:faast packaging, and on EZ:faast kit components.	
	Symbol for "In Vitro Diagnostic Medical Device"
	Symbol for "Manufacturer"
	Symbol for "Authorised Representative In The European Community"
	Symbol for "Use By" and/or "Expiration Date"
	Symbol for "Batch Code" and/or "Lot Number"
	Symbol for "Catalogue Number"
	Symbol for "Serial Number"
	Symbol for "Flammable Substances"
	Symbol for "Irritating or Harmful Substances"
	Symbol for "Corrosive Substances"

Table of Contents

1.0 Kit Components

1.1 Reagents

Reagent	Ingredients	Volume
Reagent 1 Internal Standard Solution	Homoarginine 0.2 mM Methionine-d_3 0.2 mM Homophenylalanine 0.2 mM	50mL
Reagent 2 Washing Solution	N-propanol	90mL
Reagent 3A Eluting Medium Component I	Sodium Hydroxide	60mL
Reagent 3B Eluting Medium Component II	N-propanol	40mL
Regent 4 Organic Solution I	Chloroform	4 vials, 6mL each
Reagent 5 Organic Solution II	Iso-octane	50mL
SD 1, 2, & 3 Amino Acid Standard Mixtures	Please refer to section 4.4 in the manual	2 vials each, 2mL each

1.2 Suppues

Sorbent tips in racks 4x96
Sample preparation vials 4x100
Microdispenser, 20-100µL 1
Syringe, 0.6mL 10
Syringe, 1.5mL 10
EZ:faast AAA-MS HPLC Column 1
Autosampler vials with inserts 4x100
User Manual 1
EZ:faast Demo Video and Reference CD 1

1.3 Materials Required but Not Supplied In Kit

- 100µL-1mL pipette (SoftGrip™ pipette [Phenomenex P/N AH0-5968] or equivalent)
- 30-300µL pipette (SoftGrip™ pipette [Phenomenex P/N AH0-5967] or equivalent)
- 10-100µL pipette (SoftGrip™ pipette [Phenomenex P/N AH0-5966] or equivalent)
- Pipette tips (Phenex™ [Phenomenex P/N AH0-5917 (200µL) and AH0-5920 (1mL)] or equivalent)
- Vortex
- Vials of an appropriate volume, with caps (see section 3.2)
- Pasteur pipettes for sample transfer (see section 3.3 step 14)
- Container for proper waste disposal
- Reagents and supplies for Protein Hydrolysis

2.0 Overview

2.1 Overview

The EZ:faast amino acid analysis procedure consists of a solid phase extraction step followed by a derivatization and a liquid/liquid extraction; derivatized samples are quickly analyzed by liquid chromatography-mass spectrometry. The solid phase extraction is performed via a sorbent packed tip that binds amino acids while allowing interfering compounds to flow through. Amino acids on sorbent are then extruded into the sample vial and quickly derivatized with reagent at room temperature in aqueous solution. Derivatized amino acids concomitantly migrate to the organic layer for additional separation from interfering compounds. Organic layer is then removed, evaporated, and re-dissolved in aqueous mobile phase and analyzed on a LC/MS system. Total sample preparation time takes around 8 minutes and analysis is performed in around 12 minutes for a total start to finish time of around 20 minutes.

A video included with this kit demonstrates the simplicity of the procedure. Please be aware that some sample preparation steps described in the video may be different than what is described in this users manual. Please use the video as a general guide, but follow the exact steps and sequence described in this manual.

2.2 Free Amino Acids in Biological Samples

The EZ:faast method has been developed for the analysis of more than 60 aliphatic and aromatic amino acids or related compounds (Table 1). Further amino acids may be analyzed with this kit. A brief adjustment of chromatographic and MS conditions may be required. Please contact your Phenomenex technical consultant for method modifications and other LC and GC amino acid kits.

Table 1. Comprehensive list of amino acids and related compounds prepared by EZ:faast for LC/MS analysis (included **internal standards** listed in bold)

Chemical Name	Abbreviation	Alternate Abbreviation	t_R, min	AA MW	Derivative MW+1	MS^2	LOD* (S/N=3) pmol/mL
Ethanolamine			2.7	61.1	148	62, 88, 106	
Pyroglutamic acid	pGLU		2.6	129.1	172	130	
Arginine	ARG		2.7	174.2	303	70, 156, 286	2
Homoarginine	**HARG**		**2.9**	**188.2**	**317**	**84, 128, 170**	**NA**
Glutamine	GLN	Q	3.3	146.2	275	172, 84, 215	5
Citrulline	CIT		3.4	175.2	304	156, 113, 287	
Anserine	ANS		3.5	240.1	369	309, 212	2
Methionine sulfoxide			3.5	165.2	294	234, 142	
Serine	SER	S	3.7	105.1	234	146, 174, 216	5
Asparagine	ASN	N	3.8	132.1	243	157, 115, 201	10
Proline-hydroxyproline (dipeptide)	PHP		3.8	228.2	357	156, 184, 297	2
Theanine	THE		3.8	174.2	303	243, 215	
3-Methyl-histidine	3MHIS		3.8	169.2	298	210, 256	5
1-Methyl-histidine	1MHIS		3.8	169.2	298	96, 196	5
4-Hydroxyproline	HYP		4	131.1	260	172, 157, 200	5
Glycine	GLY	G	4.3	75.1	204	144, 118, 162	5
Glycine-proline (dipeptide)	GPR		4.3	172.2	301	158, 241	1

Chemical Name	Abbreviation	Alternate Abbreviation	t_R, min	AA MW	Derivative MW+1	Derivative MS^2	LOD* (S/N=3) pmol/mL
Threonine	THR	T	4.3	119.1	248	160, 188, 230	10
Methionine sulfone			4.4	181.2	310	250, 222, 142	
Alanyl-alanine (dipeptide)	ALA-ALA		4.5	160.2	289	229	
Methionine sulfoximine			5	180.2	395	230, 188	
β-Alanine	βALA		5	89.1	218	98, 116, 158	
Alanine	ALA	A	5.1	89.1	218	130, 158, 88	20
δ-Hydroxylysine	HLY		5.4	162.1	377	317, 125, 359	100
4-Aminobutyric acid	GABA		5.6	103.1	232	172, 130	5
Histamine	HA		5.7	111.1	284	198, 138	
β-Aminobutyric acid	βABA		5.8	103.1	232	130, 172, 88	
Sarcosine	SAR		5.8	89.1	218	88, 158, 116	10
β-Aminoisobutyric acid	βAIBA		6.1	103.1	232	172, 130	5
α-Aminobutyric acid	ABA		6.5	103.1	232	172, 130, 144	1
2,4-diaminobutyric acid	DABA		6.7	118.1	333	273	
Ornithine	ORN	O	6.9	132.1	347	287, 156, 227	1
Carnosine			6.9	226.2	441	381, 284, 353	
Lysine-alanine (dipeptide)	LYS-ALA		6.9	217.2	432	317, 301, 170	
Methionine-*d3*	**Met-*d3***		**6.9**	**152.2**	**281**	**193, 221, 142**	**NA**
Methionine	MET	M	7	149.2	278	190, 218, 142	1
Proline	PRO	P	7.1	115.1	244	156, 114, 184	1
S-Pyridylethyl-cysteine			7.5	226.3	355	106	
Lysine	LYS	K	7.7	146.1	361	301, 170	0.1
Aspartic acid	ASP	D	7.8	133.1	304	216, 130, 244	1
Histidine	HIS	H	7.8	155.1	370	196, 110, 284	1
Thiaproline	TPR		7.9	133.2	262	174, 88, 202	10
Seleno-methionine	Se-MET		8	196.1	326	238	
Homoserine	HSER		8.1	119.1	230	188	
Valine	VAL	V	8.2	117.1	246	158, 116, 186	1
Aspartame			8.3	294.3	423	363, 391	
Glutamic acid	GLU		8.3	147.1	318	172, 258, 230	1
Tryptophan	TRP	W	8.5	204.2	333	245, 273, 230	2
Carboxymethyl-cysteine			8.7	179.2	350	290	
Ethionine	ETH		8.8	163.2	292	204	
γ-Glutamyl-ε-lysine (dipeptide)	GLU-LYS		8.8	275.3	532	472, 412	
α-Aminoadipic acid	AAA		9.5	161.2	332	244, 185, 272	
Leucine	LEU	L	10	131.2	260	172	2
Phenylalanine	PHE	F	10.1	165.2	294	206, 120	1
allo-Isoleucine	alLE		10.5	131.2	260	172, 200	
Isoleucine	ILE	I	10.6	131.2	260	172, 130, 74	5
Adrenaline			10.6	183.2	424	338, 382, 252	
Norleucine	NLEU		10.9	131.2	260	172, 200	
Cysteine	CYS		10.9	121.2	336	190, 248, 276	
Aspartame acid	ASP-PHE		11.2	280.3	451	391	
Aminopimelic acid	APA		11.4	175.2	346	258, 198, 286	5
Arginino-succinic acid			11.4	334.2	529	383, 443	

Table 1 - (continued)

Chemical Name	Abbreviation	Alternate Abbreviation	t_R, min	AA MW	Derivative MW+1	Derivative MS^2	LOD* (S/N=3) pmol/mL
Pipecolic acid	HPRO		11.5	129.1	258	170, 198, 128	
Dopamine	DA		11.5	153.1	412	266, 326	
Lysinoalanine	LAL		11.7	233.2	430	370, 342	
Cystathionine	CTH		11.9	222.3	479	230, 188, 419	5
4-Aminobenzoic acid	PABA		11.9	137.1	266	224	
Homocysteine	HCYS		12	135.2	350	204, 290	
Homophenylalanine	**HPHE**		**12.2**	**179.2**	**308**	**220, 117, 104**	**NA**
Cystine	C-C	C	12.5	240.3	497	248, 437, 306	5
Tyrosine	TYR	Y	13.3	181.2	396	136, 308, 336	10
Cysteine-Homocysteine (dipeptide)	C-HC		13.3	320.1	511	262, 451	
3-Hydroxy-tyrosine**	DOPA		13.5	197.2	498	352, 412, 438	
Homocystine**	HC-CH		13.8	268.3	525	262, 88, 465	
Seleno-cystine**	Se-C		14.3	334.1	593	296, 371	
Dimethylarginine***	SDMA		10.0***		202.2	331	98
Dimethylarginine***	ADMA		11.2***		202.2	331	98

**LODs were determined for amino acids included in the standard mixtures provided with the kit*
***extended gradient time required*
**** alternate chromatographic conditions required*

2.3 Storage and Stability

Store Reagents 1, 3B and 4 at 4°C. Store amino acid standard solutions in the freezer. All other components may be stored at room temperature. For your convenience, the bottom of the reagent box has been designed as a tray that can be easily lifted from the workstation and placed in the refrigerator when the kit is not in use for an extended period of time.

All components are guaranteed for 12 months or more (see label on bottle/vial) from the date of purchase when stored at recommended temperatures and used as described in this manual. Please review the Instruction Manual included with the Drummond® Dialamatic Microdispenser for recommended usage and warranty information. Please observe recommendations for solvent bottle handling and syringe cleaning in Section 6.0 of this manual.

2.4 Safety

Although the concentration of all toxic components in any of the reagent bottles is low, for safety reasons the sample preparation station should be placed in an exhaust hood and protective gloves and goggles should be worn. When working with biological fluids, please take any necessary precautions to prevent infection with blood borne pathogens. Appropriate bio-safety precautions and disposal of bio-hazardous wastes should be followed.

3.0 Sample Preparation Procedure

3.1 Setup

The EZ:faast kit packaging has been designed as an efficient workstation. It holds a reagent tray, a vial rack, a pipette rack and a section for sorbent tips and vials. To speed up sample preparation it is recommended that the workstation be arranged as shown in figure 1a. By following directions and markings on the reagent box by breaking it along perforations, it can be transformed into a reagent tray. When the kit is not in use for several days, the reagent tray (figure 1b) may be conveniently removed and placed in the refrigerator.

Workstation Arrangement - (Figure 1)

To speed up sample preparation it is recommended that the workstation be arranged as shown below.

Figure 1a

Figure 1b

3.2 Preparing the Eluting Medium

The volume of prepared Eluting Medium depends upon the number of samples to be analyzed during the day (200µL/sample). The eluting medium should be prepared fresh each day:

1. Use capped vials of appropriate size (not included) for preparation of the Eluting Medium
2. Combine 3 parts Reagent 3A (Eluting Medium Component I) with 2 parts Reagent 3B (Eluting Medium Component II) in an appropriate sized vial (see Table 2 below, for reagent volumes based on number of samples). Mix briefly.
3. Store prepared eluting medium during the day at room temperature. Discard any unused mixture at the end of the day.

Table 2 - For your convenience check the table below to determine the volume of Eluting Medium components needed depending on your number of samples:

Number of Samples	Reagent 3A Eluting Medium Component I	Reagent 3B Eluting Medium Component II
2	300µL	200µL
4	600µL	400µL
7	900µL	600µL
12	1.5mL	1.0mL
14	1.8mL	1.2mL
19	2.4mL	1.6mL
24	3.0mL	2.0mL
29	3.6mL	2.4mL
34	4.2mL	2.8mL
39	4.8mL	3.2mL
44	5.4mL	3.6mL
49	6.0mL	4.0mL

3.3 Sample Preparation by SPE and Derivatization

Prepare Eluting Medium first; refer to section 3.2 for preparation protocol. The freshly prepared Eluting Medium vial may be placed in one of the empty slots in the reagent tray.

1. For each sample, line one glass sample preparation vial in the vial rack (Figure 2). Be aware of some variability in vial opening and sorbent tip dimensions, which may prevent the tip from reaching to the bottom of the sample preparation vial.

Note: *drops of solvent in SPE tip or spilled sorbent particles will not affect the precision of the assay in any way.*

Glass Vial Line Up - (Figure 2)

For each sample, line up one sorbent tip and one glass sample preparation vial in the vial rack.

Figure 2

2. Pipette 100µL sample (serum, plasma, urine or other), and 100µL Reagent 1 (Internal Standard Solution) into each sample preparation vial.

Note: *Samples with amino acid concentrations higher than 10mmol/L (10µmol/mL; e.g. dark colored urine) should be analyzed by pipetting only 50µL (or 25µL) sample in the sample preparation vial instead of 100µL. Concentrations recorded as a result of the LC analysis will be half (one quarter) of the actual concentrations for these samples. Conversely, when low concentrations of amino acids have to be quantified, the volume of sample to be prepared should be 200µL or more. The total amount of amino acids present in the sample to be loaded onto the SPE tip should not exceed 1.2µmols!*

Caution: The pH of biological samples is usually around 7. After the addition of Reagent 1 (Internal Standard) the mixture has the correct pH for successful loading onto the SPE tip as described in the next step. With other samples make sure that the sample + Reagent 1 mixture has a pH between pH 1.5 and pH 6.0!

3. Attach a sorbent tip to a 1.5mL syringe and loosen the syringe piston; immerse the tip and let the solution in the sample preparation vial pass through the sorbent tip by SLOWLY pulling back the syringe piston, in SMALL steps.

Caution: Do not quickly pull back the piston. Try to take at least one minute to pass low viscosity sample (such as urine or standard) through the sorbent tip. For very viscous samples like concentrated plasma, 200µL of water can be added to ease the sample transfer through the sorbent. The syringe should be capable of drawing all sample, and subsequent wash reagent into the barrel. Watch as the liquid accumulates inside the syringe barrel and move the piston only as the accumulation slows down. Urine passes relatively fast through the sorbent bed, while serum and plasma pass much slower. If you run out of piston range, detach the sorbent tip, expel the solution from the syringe barrel, then reattach the sorbent tip and proceed with sample preparation.

Note: *the sorbent tip should stay in the sample preparation vial through steps 3-9 (see figure 3) even while dispensing reagents. In case the sorbent tip cannot reach to the bottom of the vial, tilt the vial to about 45°, push the tip into the vial gently, and proceed with the SPE step.*

4. Pipette 200µL Reagent 2 (Washing Solution) into the same sample preparation vial.
5. Pass the solution SLOWLY through the sorbent tip and into the syringe barrel. Drain the liquid from the sorbent bed by pulling air through the sorbent tip.
6. Detach the sorbent tip, and leave it in the sample preparation vial, then discard the liquid accumulated in the syringe.

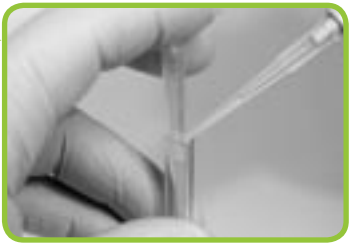
Note: *save the syringe, as it will be reused with many other samples. For convenience place it into the pipette rack.*

7. Pipette 200µL Eluting Medium (prepared fresh each day, section 3.2) into the same sample preparation vial.
8. Pull back the piston of a 0.6mL syringe halfway up the barrel and attach the sorbent tip used in steps 3-5.

Keep the Sorbent Tip in the Vial - (Figure 3)

Keep the sorbent tip in the sample preparation vial through steps 3-9, even while dispensing Reagent 2 at step 4 and Eluting Medium at step 6.

Figure 3



9. Wet the sorbent with Eluting Medium; watch as the liquid rises through the sorbent particles and stop approximately when the liquid reaches the filter plug in the sorbent tip.
10. Eject the liquid and sorbent particles out of the tip and into the sample preparation vial. Repeat step 8 and 9 until all sorbent particles in the tip are expelled into the sample preparation vial. Only the filter disk should remain in the empty tip; see Figure 4. Discard the empty tip. Keep the syringe as it can be reused with many other samples.
11. Using the adjustable Drummond Dialamatic Microdispenser (included) transfer 50µL Reagent 4 into the sample preparation vial.

Caution: Avoid cross-contamination by not touching the inner wall of the sample vial with the tip of the Microdispenser. The piston will ensure proper transfer of liquids into the vial without the need of touching the vial wall. Use the same Microdispenser with both Reagents 4 and 5. There is no need to change Microdispenser tips between uses, or to wash the dispenser between uses of Reagent 4 and 5.

Warning: *Do not use regular pipettes and tips with Reagent 4 and 5 as they will contaminate the sample! Use the included Microdispenser for Reagents 4 and 5 ONLY!*

Note: *for all subsequent sample preparation steps use a vortex mixer set in the touch (pulse) mode (to about 80% of max speed) for any mixing operations.*

12. Emulsify the liquid in the vial by repeatedly vortexing for about 5-8 seconds. During vortexing hold the sample vial firmly between fingers, and keep it straight as you push it onto the vortex plate. Do not let vial wobble, otherwise liquid may come out of the vial. Allow reactions to proceed 1 minute or more.

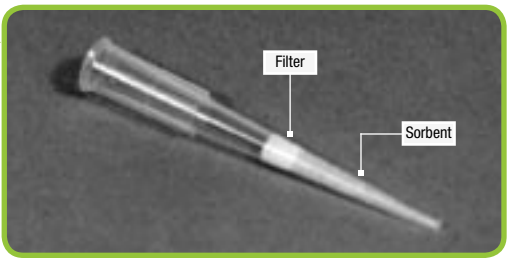
Note: *a longer reaction time than 1 minute after step 11 and 12, or later, 1 minute after step 13, does not affect results.*

13. Re-emulsify the liquids in the vial by vortexing again for about 5 seconds. Allow the reaction to proceed for one additional minute or more.

Sorbent Tip - (Figure 4)

Wet the sorbent with Eluting Medium and stop before it gets to the filter then eject the liquid and sorbent particles out of the tip.

Figure 4



14. Transfer with the Microdispenser 100µL Reagent 5 (2 x 50µL for convenience), and mix for about 5 seconds. Let the reaction proceed for one more minute.
15. Transfer part of the (upper) organic layer (about 50µL) into an autosampler vial (included) using a Pasteur pipette, and evaporate to dryness in a gentle stream of nitrogen at room temperature. Avoid the transfer of aqueous layer along with the organic layer! Do not leave samples in the nitrogen stream for more than 10 minutes! Re-dissolve in 70-100 µL mixture of HPLC mobile phase components (10mM ammonium formate in water: 10mM ammonium formate in methanol 1:2, v/v). Transfer reconstituted sample into an insert (included), and place insert into the same autosampler vial. The sample is ready for LC/MS analysis.

3.4 Optimizing Sample Preparation Time

For experienced users, sample preparation proceeds in 7-8 minutes per sample. This process can be further improved by preparing up to ten samples at a time. For example, at step 2 dispense Reagent 1 (and at later steps all other reagents) in ten vials successively, using the same pipette tip. At step 9, after dispensing Reagent 4, vortex 2-3 vials simultaneously. During each 1 minute wait at steps 10-12 prepare autosampler vials for sample transfer.

4.0 LC/MS Analysis

4.1 Column for EZ:faast LC/MS Analysis:

The HPLC column dimension included in the kit should be based on the flow rate most compatible with your LC/MS system:

- Flow 0.5 mL/min: EZ:faast AAA-MS column 250 x 3.0 mm
- Flow 0.25mL/min: EZ:faast AAA-MS column 250 x 2.0 mm

Column should be equilibrated by running a blank gradient. Column can be stored in mobile phase when not in use.

Note: *because of column length, and the use of a sorbent with small particle size and a mobile phase of high viscosity (methanol/water), the expected column backpressure is 200-220 bar (2,900-3,200 psi). The column supplied with the kit will tolerate this backpressure very well.*

4.2 Instrument Settings:

HPLC

Mobile phase:	A: 10mM Ammonium formate in water B: 10mM Ammonium formate in methanol	
Gradient:	00.00min 13.00 13.01 17.00 Re-equilibration time may vary between 4 and 7 minutes depending on the gradient delay time of the HPLC instrument.	68%B 83%B 68%B 68%B
Flow rate:	0.50mL/min. for 3.0mm ID column 0.25mL/min. for 2.0mm ID column	
Column temperature:	35°C	
Injection volume:	1µL	

MS

Either ESI or APCI may be used

Mode:	Positive Ion
Scan range:	100-600 m/z
ESI ion source temperature:	365°C (Bruker); 425°C (AB API 3000)
APCI ionization chamber temperature:	450°C

4.3 Tuning the Mass Spectrometer

Some mass spectrometers require a concentrated calibration solution for tuning the instrument (if not then calibration solution III {see section 4.5} can be used). To prepare the concentrated solution, dispense 200µL aliquots of SD1, SD2, SD3 and Reagent 1 into each of two sample vials (individual standard solutions can be omitted if not relevant). Perform the SPE and derivatization steps to each vial as described by the EZ:faast procedure (section 3). Transfer the organic layers from the two sample vials into one autosampler vial and evaporate to dryness with a nitrogen stream. Reconstitute in 200µL of 1:2 mobile phase A:B mixture and use for tuning the mass spectrometer.

Best results can be achieved by tuning the mass-spectrometer for all analytes of interest in your particular assay. Triple-quadrupole mass-analyzers allow tuning for a large number of ions if dwell-times are chosen judiciously. Nevertheless, some mass spectrometers will not allow for concomitant tuning for all ions as required for amino acid profiling. This impediment can be easily overcome by creating time segments (periods) in the run file where a selected group of ions are analyzed within each segment. This use of segments allows for optimal tuning for a large number of desired amino acids. It is recommended that ion-trap mass-analyzers be tuned at least for the analytes suggested in the table below.

A suggested breakdown of the MS analysis into three segments looks as follows:

Time	Suggested Tune AA	AA in Range	AA at End of Range
0-4.8 min	ASN and GLN	R,Q,S,N,G,T	T
4.8-9.3 min	MET and ASP	A,M,P,K,D,H,V,E,W	E(W)
9.3-14 min	LEU and C-C	L,F,I,C-C,Y	Y

A suggested breakdown of the MS analysis into six segments looks as follows:

Time	Suggested Tune AA	AA in Range	AA at End of Range
0-4.8 min	GLN and ASN	R,Q,S,N,G,T	T
4.8-6.5 min	ALA	A	A
6.5-7.7 min	MET and PRO	M,P	P
7.7-9.3 min	ASP and VAL	K,D,H,V,E,W	E(W)
9.3-10.7 min	LEU and PHE	L,F,I	I
10.7-14 min	C-C and TYR	C-C,Y	Y

The segment time limits and amino acids to tune for may be different depending on instrument and application. HPLC pumps having larger gradient delay times will produce longer retention times and segments must be adjusted accordingly. For your convenience, we have included methods for Applied Biosystems API3000 LC/MS/MS and Bruker Daltonics Esquire2000 LC/MS instruments on the reference CD included with this kit. To use any of these files copy it into the appropriate folder in your software. Text file versions of the method files illustrate instruments settings, including tune parameters.

Note: *With some mass-spectrometers, sodated molecular ions (derivative MW+22) are more prevalent than protonated ions (e.g. m/z 330 maybe observed for HPHE, instead of m/z 308). In such cases it is beneficial to acidify the sample with formic acid by reconstituting the sample prepared for tuning in 0.1% formic acid and 10mM ammonium formate in both methanol and water (2:1, v/v).*

4.4 Calibration Standards

For quantitation purposes, prepare aliquots of amino acid standard mixtures following the Sample Preparation by SPE and Derivatization procedure described in this manual in Section 3.3. Standard mixtures should be stored in the freezer as some amino acids are not stable in solution.

Three vials of different standard mixtures are included in the kit:

SD1: 27 amino acids in 0.05N HCl, 200 nmoles/mL each, as follows:

AAA	C-C	HYP	3MHIS	THR
ABA	CIT	ILE	ORN	TYR
ALA	GABA	LEU	PHE	VAL
ARG	GLU	LYS	PRO	
ASP	GLY	MET	SAR	
ßAIB	HIS	1MHIS	SER	

SD2: Complementary amino acids not stable in acidic solution, 200 nmoles/mL each, as follows:

ASN	GLN	TRP

SD3: Complementary urine amino acids and dipeptides, 200 nmoles/mL each, as follows:

APA	CTH	GPR
HLY	PHP	TPR

4.5 Calibration Procedure

Use the following standard amino acid mixtures and make duplicate injections of each to generate the desired calibration:

Calibration Solution

I. 10µL of SD1 solution, plus 10µL of SD2 (and/or SD3 depending on your application), plus 100µL Internal Standard solution (calibration level I: 20 nmols/mL)

II. 50µL SD1+ 50µL SD2 (and/or SD3)+ 100µL IS (calibration level II: 100 nmols/mL)

III. 100µL SD1+ 100µL SD2 (and/or SD3)+ 100µL IS (calibration level III: 200 nmols/mL)

The concentration of each internal standard (IS)--HARG, MET-d3 and HPHE-- in calibrators and samples prepared for chromatographic analysis is 200 nmoles/mL. While the use of the ideal internal standard will vary based on instrument and application, we recommend using HARG as the internal standard for ARG and CIT; MET-d3 as the internal standard for GLN through TRP (early and middle eluting amino acids); and HPHE for LEU through TYR (late amino acids). Other amino acids can be added and used as internal standards based on the application.

4.6 Calculation of Analytical Results

Calculations are performed by the Data Analysis portion of the software controlling the analytical instrument (liquid-chromatograph). Calculations and calibration are based on internal standard(s). Results are reported in the units entered for internal standard(s) and analyte levels in calibration mixtures.

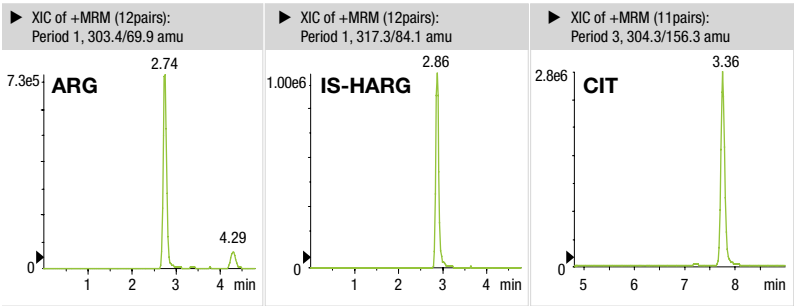
Note: *nmols/mL are equivalent to µmols/L.*

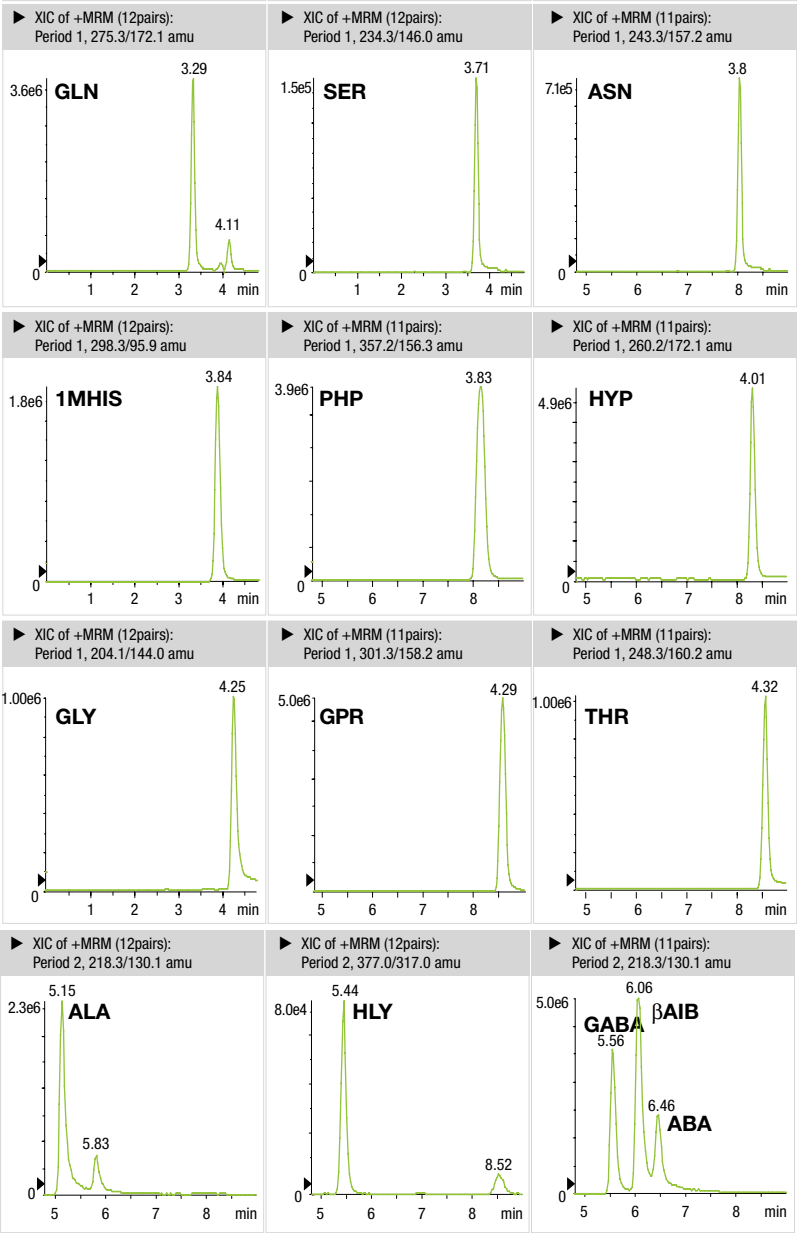
Remember: the SD1, SD2 and SD3 vials should be placed in the freezer after use! Allow standards to reach room temperature before use.

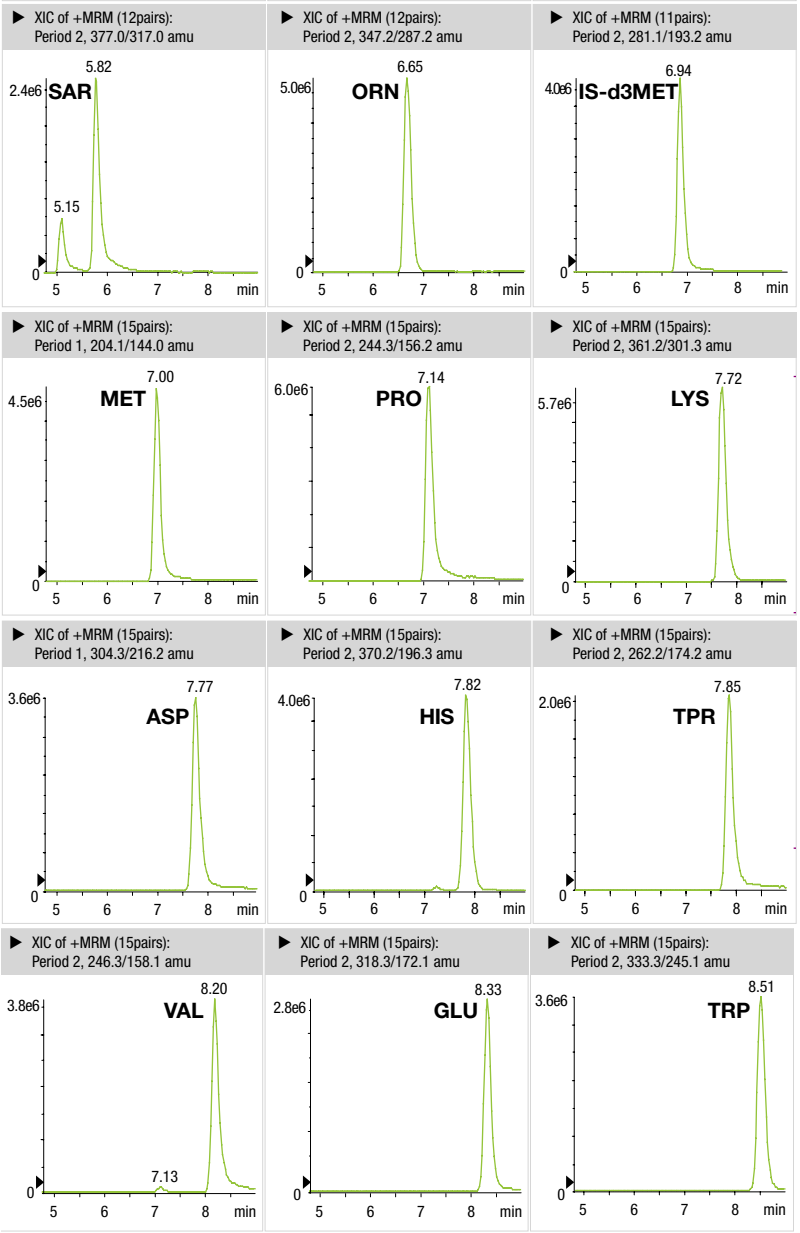
Extracted Ion Chromatograms for the amino acids included in standard mixtures provided with the kit (in order of elution; at 200nmol/mL, each):

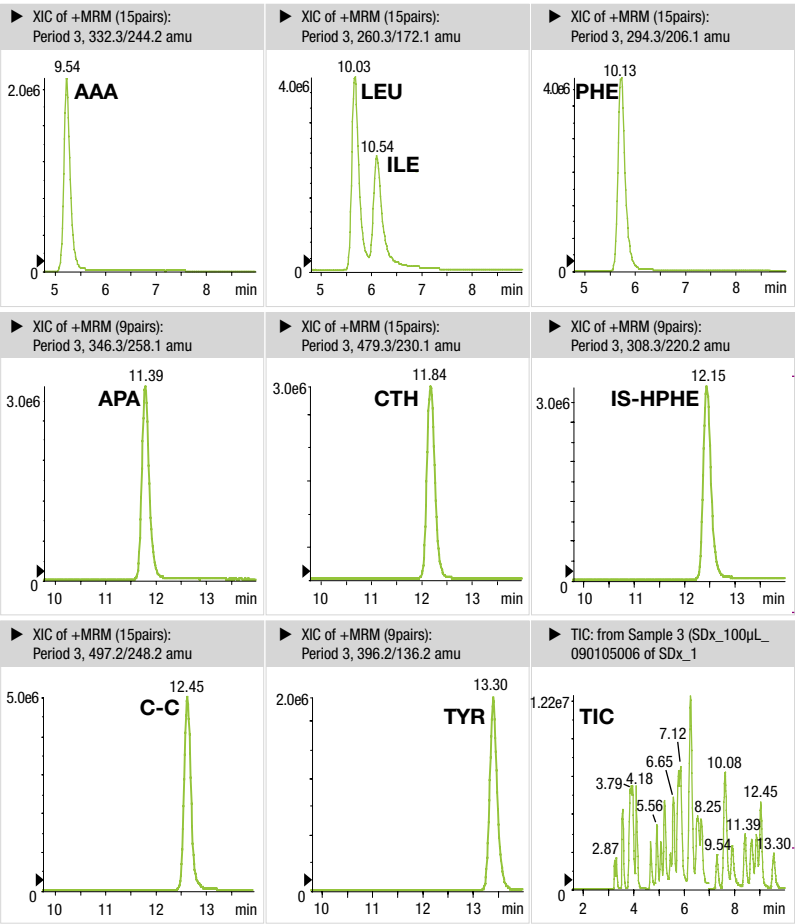
5.0 Sample Storage and Stability

Some amino acids are chemically unstable in physiological fluids (e.g., progressive decline of plasma glutamine and cystine in time), and also in standard mixtures. Keep samples and standard mixtures in the freezer. Old amino acid standard mixtures and mixtures which have not been stored properly should not be used for instrument calibration. Order fresh mixtures




XIC of +MRM (12pairs): Period 1, 275.3/172.1 amu
GLN
3.29
4.11
XIC of +MRM (12pairs): Period 1, 234.3/146.0 amu
SER
3.71
XIC of +MRM (11pairs): Period 1, 243.3/157.2 amu
ASN
3.8
XIC of +MRM (12pairs): Period 1, 298.3/95.9 amu
1MHIS
3.84
XIC of +MRM (11pairs): Period 1, 357.2/156.3 amu
PHP
3.83
XIC of +MRM (11pairs): Period 1, 260.2/172.1 amu
HYP
4.01
XIC of +MRM (12pairs): Period 1, 204.1/144.0 amu
GLY
4.25
XIC of +MRM (11pairs): Period 1, 301.3/158.2 amu
GPR
4.29
XIC of +MRM (11pairs): Period 1, 248.3/160.2 amu
THR
4.32
XIC of +MRM (12pairs): Period 2, 218.3/130.1 amu
ALA
5.15
5.83
XIC of +MRM (12pairs): Period 2, 377.0/317.0 amu
HLY
5.44
8.52
XIC of +MRM (11pairs): Period 2, 218.3/130.1 amu
GABA
5.56
βAIB
6.06
ABA
6.46
min


XIC of +MRM (12pairs): Period 2, 377.0/317.0 amu
SAR
5.82
5.15
2.4e6
0
5 6 7 8 min
XIC of +MRM (12pairs): Period 2, 347.2/287.2 amu
ORN
6.65
5.0e6
0
5 6 7 8 min
XIC of +MRM (11pairs): Period 2, 281.1/193.2 amu
IS-d3MET
6.94
4.0e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 1, 204.1/144.0 amu
MET
7.00
4.5e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 244.3/156.2 amu
PRO
7.14
6.0e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 361.2/301.3 amu
LYS
7.72
5.7e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 1, 304.3/216.2 amu
ASP
7.77
3.6e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 370.2/196.3 amu
HIS
7.82
4.0e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 262.2/174.2 amu
TPR
7.85
2.0e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 246.3/158.1 amu
VAL
8.20
7.13
3.8e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 318.3/172.1 amu
GLU
8.33
2.8e6
0
5 6 7 8 min
XIC of +MRM (15pairs): Period 2, 333.3/245.1 amu
TRP
8.51
3.6e6
0
5 6 7 8 min



from Phenomenex (see ordering information on page 17 of this manual).

Samples prepared for LC-MS analysis following the procedure outlined in this manual may be stored for several days in a freezer before analysis. Storing dry samples is preferable to storing reconstituted samples. Dry down the organic solvent as described in section 3.3 step 15, cap vials, and place them in the freezer. For longer storage we recommend that the organic layer be desiccated with anhydrous sodium sulfate before solvent removal, vials be capped and placed in the freezer. Since sample preparation is expeditious with this procedure we recommend analyzing samples prepared freshly. Samples prepared during the day may be left on the autosampler tray, at room temperature, to be analyzed during the night or the next day.

6.0 Cleaning and Care of Supplies

The Drummond® Dialamatic Microdispenser should be flushed with isopropanol: acetone (approx. 1:1) at the end of the day. Please review the Drummond Microdispenser users manual for further care and use notes. The same organic mix is recommended as wash for both manual syringes and autosamplers. Plastic syringes used for SPE can be cleaned by flushing with a propanol:water (1:2, v/v) mixture. Always tightly cap the reagent bottles when not in use in order to avoid solvent evaporation and alteration of reagent composition. Cover the racks holding sorbent tips when not in use to prevent contamination.

7.0 Quality Assurance

All components of the EZ:faast Amino Acid Analysis kit are subjected to rigorous quality control testing. These measures help to ensure the best results. If poor results occur, please contact your Phenomenex technical consultant or distributor.

8.0 Product Limitations

Phenomenex Analyte Specific Reagent products are not intended for clinical use. Because they are not intended for clinical use, no claim or representation is made or intended for their clinical use (including, but not limited to diagnostic, prognostic, therapeutic or blood banking). It is the user's responsibility to validate the performance of Phenomenex products for any particular use, since the performance characteristics are not established. Phenomenex products may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by the Clinical Laboratory Improvements Amendments of 1988 (CLIA '88) regulation in the U.S. or equivalent in other countries.

Trademarks

ordering information

EZ:faast™ Kit

Each kit includes: a EZ:faast AAA LC column (or ZB-AAA GC column and liners), sample prep and derivatization reagents, sample prep vials, AA standard mixtures, SPE sorbent tips, autosampler vials with inserts come with MS kits, Microdispenser for Reagents 4 and 5, and demo video.

Description	Order No.	Unit
GC-FID Free (Physiological) Amino Acid Analysis Kit	KG0-7165	ea
GC-MS Free (Physiological) Amino Acid Analysis Kit	KG0-7166	ea
GC-FID Protein Hydrolysate Kit	KG0-7167	ea
GC-MS Protein Hydrolysate Kit	KG0-7168	ea
LC/MS Free (Physiological) Amino Acid Analysis Kit with 250 x 2.0mm column	KH0-7337	ea
LC/MS Free (Physiological) Amino Acid Analysis Kit with 250 x 3.0mm column	KH0-7338	ea
LC/MS Protein Hydrolysate Kit with 250 x 2.0mm column	KH0-7339	ea
LC/MS Protein Hydrolysate Kit with 250 x 3.0mm column	KH0-7340	ea
GC Free (Physiological) Amino Acid Standards (SD1, SD2 & SD3) 2mL/vial x 2	AG0-7184	ea
GC Protein Hydrolysate Standard (SD) 2mL/vial x 2	AG0-7263	ea
LC/MS Free (Physiological) Amino Acid Standards for LC (SD1, SD2, & SD3) 2mL/vial x 2	AL0-7500	ea
LC/MS Protein Hydrolysate Standard (SD) 2mL/vial x 2	AL0-7501	ea

Phenex™ Vials

This universal vial can be used in any autosampler that utilizes a 12 x 32mm vial. It may be used in place of crimp top and snap ring top vials. Eliminates the need of stocking many different style vials. The top screws down in 1/3 turn and eliminates the chore of crimping, de-crimping and snapping caps on. Cap comes with a bonded-in septa that eliminates septa slipping into vials. Vials and caps with bonded-in septa come in one convenient kit pack.

Description	Order No.	Unit
Clear wide mouth vial, cap and septa kit pack with:		
Rubber/PTFE septa	AH0-4610	1000/pk
Silicone/PTFE septa	AH0-4613	1000/pk
PTFE/Silicone/PTFE septa	AH0-4616	1000/pk
Amber wide mouth vial, cap and septa kit pack with:		
Rubber/PTFE septa	AH0-4619	1000/pk
Silicone/PTFE septa	AH0-4622	1000/pk
Clear wide mouth vial, cap with pre-slit septa:		
Silicone/PTFE septa	AH0-7507	1000/pk

EZ:faast™
User's Manual

EZ:faast - Free (Physiological) Amino Acid Analysis by LC-MS

Quick Reference Guide

Summary of Procedure:

1. For each sample line up one glass sample preparation vial in the vial rack.
2. Pipette 100µL sample (serum, plasma, urine or other; see section 3.3.2) and 100µL Reagent 1 into each sample preparation vial.
3. Attach a sorbent tip to a 1.5mL syringe; pass the solution in the sample preparation vial through the sorbent tip by **slowly** pulling back the syringe piston.
4. Pipette 200µL Reagent 2 (Washing Solution) into the sample preparation vial.
5. **Slowly** pass the solution through the sorbent tip and into the syringe barrel.
6. Detach the sorbent tip, and discard the liquid accumulated in the syringe.
7. Pipette 200µL Eluting Medium (prepared fresh each day, section 3.2) into the sample preparation vial.
8. Pull back the piston of a **0.6mL syringe** halfway up the barrel and attach the sorbent tip.
9. Wet the sorbent with Eluting Medium; stop when the liquid reaches the filter plug in the sorbent tip.
10. Eject the liquid and sorbent out of the tip and into the sample preparation vial. Repeat, until all sorbent particles in the tip are expelled into the sample preparation vial. Discard the empty tip.
11. Using the Drummond Dialamatic Microdispenser, transfer 50µL Reagent 4.
12. **Emulsify** by repeatedly vortexing the solution for about 5 seconds. Allow reaction to proceed for about 1 minute.
13. Vortex the solution again for a few seconds to re-emulsify the content of the vial. Allow the reaction to proceed for at least one additional minute.
14. Using the Microdispenser, transfer 100µL Reagent 5, and re-emulsify by vortexing for about 5 seconds. Let the reaction proceed for 1 minute.
15. Transfer part of the (upper) organic layer (50-100µL) with a Pasteur pipette into an autosampler vial. Avoid transferring aqueous layer along with the organic layer. Evaporate the solvent SLOWLY to dry under a gentle stream of nitrogen (max 10 min). Re-dissolve amino acid derivatives in 100µL (or less) of a mixture of LC mobile phase components A:B 1:2 (v/v). Transfer the reconstituted sample into an insert, and place the insert in the same autosampler vial. The reconstituted sample is ready for LC/MS analysis.

LC-MS Analysis

LC Settings

Mobile phase:	A: 10mM Ammonium formate in water B: 10mM Ammonium formate in methanol
Gradient:	0.00min 68%B 13.00 83%B 13.01 68%B 17.00 68%B Re-equilibrate column for 4-6 min before next injection depending on HPLC system used.
Flow rate:	0.50mL/min. for 3.0mm ID column 0.25mL/min. for 2.0mm ID column
Column temperature:	35°C
Injection volume:	1µL

MS Settings

Either ESI or APCI may be used

Mode:	Positive Ion
Scan range:	100-650 m/z
ESI ion source temperature:	365°C (Bruker); 425°C (AB API3000)
APCI ionization chamber temperature:	450°C

CE

Detach Here

3882_L3

ordering information

EZ:faast™ Kit

Each kit includes: a EZ:faast AAA LC column (or ZB-AAA GC column and liners), sample prep and derivatization reagents, sample prep vials, AA standard mixtures, SPE sorbent tips, vial rack, autosampler vials with inserts come with MS kits, Microdispenser for Reagents 4 and 5, and demo video.

Description	Order No.	Unit
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