

EZ:Faast™ AMINO ACID ANALYSIS

Patent Pending



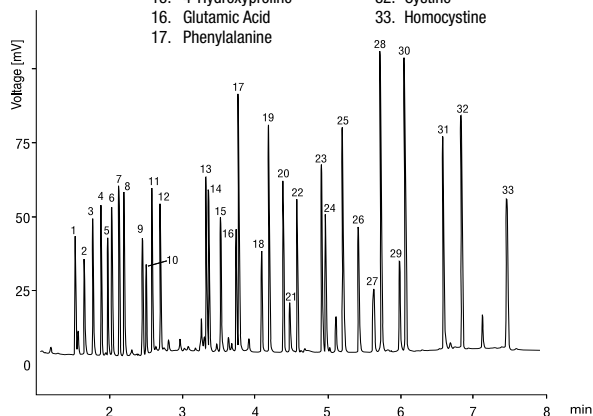
AMINO ACID ANALYSIS IN 15 MINUTES

- Physiological (free) amino acid sample prep and analysis designed for complex blood, plasma or urine matrices
- No plasma protein or urea removal required for physiological samples
- Great sample purity and improved reproducibility as most matrix components are removed during sample prep
- Method can be optimized for sulfur-containing and aromatic amino acids, along with numerous other amino acid derivatives
- One of the most economical kits with reagents and supplies for analysis of 384 samples

App ID 14168

Amino Acid Standards

Kit:	EZ:faast GC/FID Free (Physiological) Amino Acid Kit	
Order No.:	KGO-7165	
Injection:	Split 1:15 @ 250°C, 2.5µL	
Carrier Gas:	Helium 1.5mL/minute (60 kPa) @ 110°C	
Pressure Rise:	6 kPa/min	
Oven Program:	30°C/min from 110° to 320°C, hold at 320° for 1 minute	
Detector:	FID @ 320°C	
Sample:	Equimolar mixture of derivatized amino acid standards (10nmol each)	
	1. Alanine	18. α-Amino adipic acid
	2. Glycine	19. Cysteine
	3. α-Aminobutyric acid	20. α-Aminopimelic acid
	4. Valine	21. Glutamine
	5. β-Aminoisobutyric acid	22. Homocysteine
	6. Norvaline	23. Ornithine
	7. Leucine	24. Glycine-proline (dipeptide)
	8. Isoleucine	25. Lysine
	9. Threonine	26. Histidine
	10. Serine	27. Hydroxylysine (2 isomers)
	11. Proline	28. Tyrosine
	12. Asparagine	29. Proline-hydroxyproline (dipeptide)
	13. Aspartic Acid	30. Tryptophan
	14. Methionine	31. Cystathionine
	15. 4-Hydroxyproline	32. Cystine
	16. Glutamic Acid	33. Homocystine
	17. Phenylalanine	



Analyze Over 50 Amino Acids*

Over 50 aliphatic and aromatic amino acids as well as dipeptides and amines can be analyzed using EZ:Faast™. Please contact Phenomenex for other amino acid analysis protocols. Amino acids presented in order of elution.

Abbreviation	Alternate Abbreviation	Chemical Name
ALA	A	Alanine
SAR	—	Sarcosine
GLY	G	Glycine
ABA	—	α-aminobutyric acid
VAL	V	Valine
β-ALA	—	β-Alanine
β-AIB	BAIBA	β-Aminoisobutyric acid
β-ABA	—	β-Amino-n-butyric acid
NORV	—	Norvaline
LEU	L	Leucine
alILE	—	allo-Isoleucine
ILE	I	Isoleucine
HSER	—	Homoserine
NLE	—	Norleucine
THR	T	Threonine
SER	S	Serine
PRO	P	Proline
GABA	—	γ-Amino-n-butyric acid
ASN	N	Asparagine
TPR	—	Thiaproline
ASP	D	Aspartic acid
MET	M	Methionine
HYP	OHPro	4-Hydroxyproline
GLU	E	Glutamic acid
PHE	F	Phenylalanine
AAA	—	α-Amino adipic acid
CYS	C	Cysteine
PABA	—	4-Aminobenzoic acid
HCYS	—	Homocysteine
APA	—	α-Aminopimelic acid
GLN	Q	Glutamine
HA	—	Histamine
THE	—	Theanine
MET-SO	—	Methionine Sulfoxide
DABA	—	2,4-Diamino-n-butyric acid
GLY-GLY	G-G	Glycine-glycine (dipeptide)
MET-SO ₂	—	Methionine Sulfone
ORN	O	Ornithine
GPR	G-P	Glycine-proline (dipeptide)
LYS	K	Lysine
THR-ASP	T-D	Threonine-aspartic acid (dipeptide)
HIS	H	Histidine
Se-CYS	—	Selenocystine
HLY	OHlys	Hydroxylysine (2 isomers)
TYR	Y	Tyrosine
DAP	—	Diaminopimelic acid
PHP	P-OHP	Proline-hydroxyproline (dipeptide)
TRP	W	Tryptophan
NTYR	—	3-Nitrotyrosine
LYS-ALA	K-A	Lysine-alanine (dipeptide)
DA	—	Dopamine
CTH	—	Cystathionine
DOPA	—	3,4-Dihydroxyphenylalanine
C-C	(Cys) ₂	Cystine
HC-HC	(Hcys) ₂	Homocystine
ARG-SUC	—	Arginino-succinic acid
Et(OH)NH ₂	—	Ethanolamine
ETH	—	Ethionine
PA	—	Pipecolic acid

Additional Amino Acids Analyzed by LC/MS Kits

ARG	Arginine
CIT	Citrulline
SDMA	Dimethyl arginine (Symmetrical)
ADMA	Dimethyl arginine (Asymmetrical)
1MHIS	1-Methyl-histidine
3MHIS	3-Methyl-histidine

*Please contact Phenomenex if your amino acid of interest is not on the list.



EZ:Faast™ AMINO ACID ANALYSIS

Free (Physiological) Amino Acid Analysis

The EZ:Faast™ gas chromatographic analysis kit for physiological amino acids provides rapid clean-up, derivatization and analysis of amino acids from complex mixtures. The new EZ:faast method yields a full amino acid profile in 15 minutes, including the time for sample preparation.

The patent-pending sample preparation and derivatization procedure eliminates the time-consuming process of protein removal. Likewise, urea is easily removed from urine; no extra urea removal steps are required. Samples are pre-column derivatized using a procedure to form stable amino acid derivatives ready for GC/FID, GC/NPD, GC/MS or LC/MS analysis.

Protein Hydrolysates

Within 15 minutes hydrolyzed protein or peptide samples are prepared and chromatographed for rapid sequencing important for the high sample volume of synthetic protein labs or biopharmaceutical labs. EZ:faast offers an amino acid analysis kit suitable for any GC/FID, GC/NPD, GC/MS or LC/MS system.

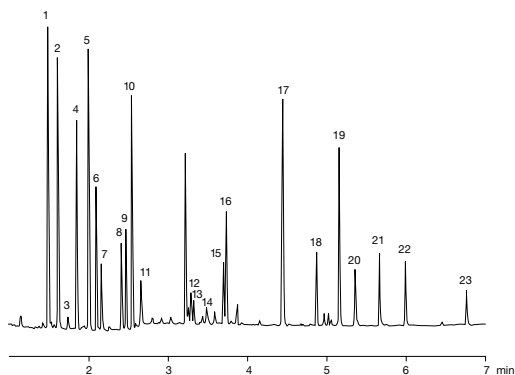
Sample preparation ensures non-hydrolyzed protein removal for more accurate analysis. Derivatized samples will not degrade rapidly.

Amino Acids in Human Serum

App ID 14169

Kit: EZ:faast GC/FID Free (Physiological) Amino Acid Kit
Order No.: KGO-7165
Injection: Split 1:15 @ 250°C, 2.5µL
Carrier Gas: Helium 1.5mL/minute (60 kPa) @ 110°C
Pressure Rise: 6 kPa/min
Oven Program: 30°C/min from 110° to 320°C, hold at 320° for 1 minute
Detector: FID @ 320°C
Sample: Derivatized amino acids in human serum (0.1mL), Norvaline is the internal standard added at a concentration of 200µmol/L

1. Alanine	13. Methionine
2. Glycine	14. 4-Hydroxyproline
3. α-Aminobutyric acid	15. Glutamic Acid
4. Valine	16. Phenylalanine
5. Norvaline (IS)	17. Glutamine
6. Leucine	18. Ornithine
7. Isoleucine	19. Lysine
8. Threonine	20. Histidine
9. Serine	21. Tyrosine
10. Proline	22. Tryptophan
11. Asparagine	23. Cystine
12. Aspartic Acid	

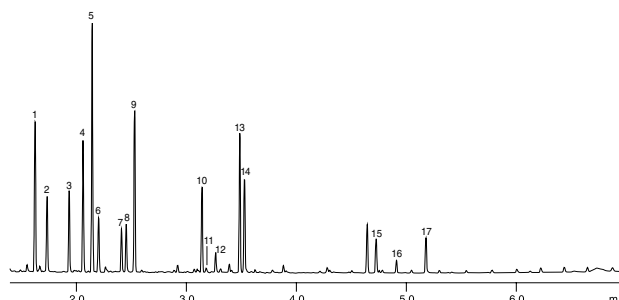


Amino Acids in Corn Meal Hydrolysate

App ID 14346

Kit: EZ:faast GC/FID Protein Hydrolysate Kit
Order No.: KGO-7167
Injection: Split 1:15 @ 250°C, 2.5µL
Carrier Gas: Helium 1.5mL/minute (60 kPa) @ 110°C
Pressure Rise: 6 kPa/min
Oven Program: 30°C/min from 110° to 320°C, hold at 320° for 1 minute
Detector: FID @ 320°C
Sample:

1. Alanine	10. Aspartic Acid
2. Glycine	11. Methionine
3. Valine	12. Hydroxyproline
4. IS=Norvaline	13. Glutamic Acid
5. Leucine	14. Phenylalanine
6. Isoleucine	15. Lysine
7. Threonine	16. Histidine
8. Serine	17. Tyrosine
9. Proline	



Архангельск (8182)63-90-72
 Астана (7172)727-132
 Астрахань (8512)99-46-04
 Барнаул (3852)73-04-60
 Брянск (4832)59-03-52
 Владивосток (423)249-28-31
 Волгоград (844)278-03-48
 Вологда (8172)26-41-59
 Воронеж (473)204-51-73
 Екатеринбург (343)384-55-89

Иваново (4932)77-34-06
 Ижевск (3412)26-03-58
 Казань (843)206-01-48
 Калининград (4012)72-03-81
 Калуга (4842)92-23-67
 Кемерово (3842)65-04-62
 Киров (8332)68-02-04
 Краснодар (861)203-40-90
 Красноярск (391)204-63-61
 Курск (4712)77-13-04
 Липецк (4742)52-20-81

Магнитогорск (3519)55-03-13
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 Мурманск (8152)59-64-93
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 Пенза (8412)22-31-16

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 Симферополь (3652)67-13-56
 Смоленск (4812)29-41-54
 Сочи (862)225-72-31
 Ставрополь (8652)20-65-13

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 Томск (3822)98-41-53
 Тула (4872)74-02-29
 Тюмень (3452)66-21-18
 Ульяновск (8422)24-23-59
 Уфа (347)229-48-12
 Хабаровск (4212)92-98-04
 Челябинск (351)202-03-61
 Череповец (8202)49-02-64
 Ярославль (4852)69-52-93



EZ:Faast™ AMINO ACID ANALYSIS

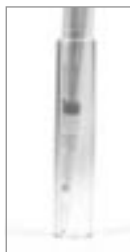
The 7 Minute Sample Preparation/ Derivatization Protocol



Step 1.
0 Minute
Pipette sample and combine internal standard
Advantage: Internal standard for improved quantitation



Step 2.
1 Minute
Pass sample through SPE sorbent tip
Advantage: No lengthy protein or urea removal



Step 3.
2 Minutes
Draw wash solution through SPE sorbent tip
Advantage: Outstanding sample purity for easy and accurate quantitation



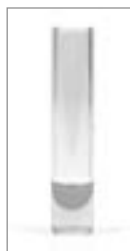
Step 4.
3 Minutes
Expel amino acids with SPE sorbent from tip
Advantage: Almost no loss of amino acids



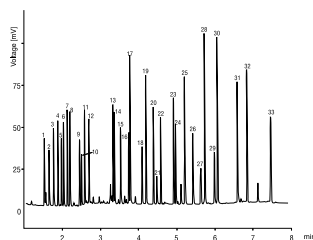
Step 5.
4 Minutes
Add derivatizing reagent
Advantage: Derivatization is complete in 2 min and can be done in aqueous phase



Step 6.
6 Minutes
Organic extraction of amino acids
Advantage: Additional purification step



Step 7.
7 Minutes
Sample preparation complete – inject onto GC or LC/MS
Advantage: Very stable derivatized sample prevents sample loss



Step 8.
15 Minutes
Analyze the chromatogram
Advantage: Full resolution of over 50 amino acids and dipeptides in a single run

No Interfering Compounds

EZ:Faast™ eliminates nearly all potential contaminants. The SPE and liquid/liquid extraction steps remove a majority of the interfering components. Additionally with the GC Kit, discrimination for non-volatile contaminants then occurs at the GC injection port. These discriminatory steps help produce chromatograms without interfering peaks from complex matrices such as plasma and urine.

Table 1:

Comparative data showing amino acid concentrations in $\mu\text{mol/L}$ from three common deproteinized plasma samples (SSA = sulfosalicylic acid; TCA = trichloroacetic acid; ORG = acetonitrile:ethanol 2:1) with a plasma sample analyzed by the GC/FID EZ:faast method. The comparative data (mean values and ranges for 12 measurements) show no significant differences between samples prepared by common protein removal procedures or by the EZ:faast method.

	Without De-proteinization EZ:faast method	SSA (Recommended for OPA-derivatized samples)	TCA	ORG (Recommended for PITC-derivatized samples)
GLY	290 (286-293)	288 (282-293)	259 (238-280)	261 (251-270)
ALA	421 (415-427)	422 (417-427)	380 (357-402)	393 (365-421)
ABA	23 (22-24)	23 (20-26)	22 (21-22)	22 (21-23)
LEU	165 (162-168)	164 (162-166)	162 (158-165)	163 (155-170)
ILE	74 (72-75)	70 (69-72)	71 (69-72)	73 (72-73)
MET	30 (29-30)	32 (31-33)	31 (30-31)	30 (29-30)
PRO	209 (207-211)	207 (204-210)	212 (208-215)	206 (197-214)
ASP	18 (17-19)	16 (15-17)	16 (14-17)	19 (18-20)

Concentration and Sensitivity

EZ:Faast™ is currently optimized for clinical concentration work at 1nmol/mL levels in biological matrices. Easy modifications can be done to look at higher and lower concentration levels from multiple sample sources.

Precision in Quantitation

The % RSD for EZ:Faast™ in looking at 1nmol/mL amino acids can be as high as $\pm 15\%$ for some problematic amino acids and dipeptides, but is $< 10\%$ for most. These % RSD values include variation due to both sample preparation and analysis, other methods usually only consider variation due to analysis.



EZ:Faast™ AMINO ACID ANALYSIS

LC/MS Kit

By taking the technology developed with the EZ:faast GC kit and applying it to LC/MS analysis, creation of the LC/MS EZ:faast amino acid analysis kit was made possible. With a rapid 8 minute clean-up and derivatization, the sample is then stable for 24 hours at room temperature or 3-4 days if refrigerated. Analysis is only 12 minutes and column re-equilibration is just 4 minutes. Below are standards analyzed by the LC/MS kit.



AMINO ACID ANALYSIS
EZ:faast

App ID 14770

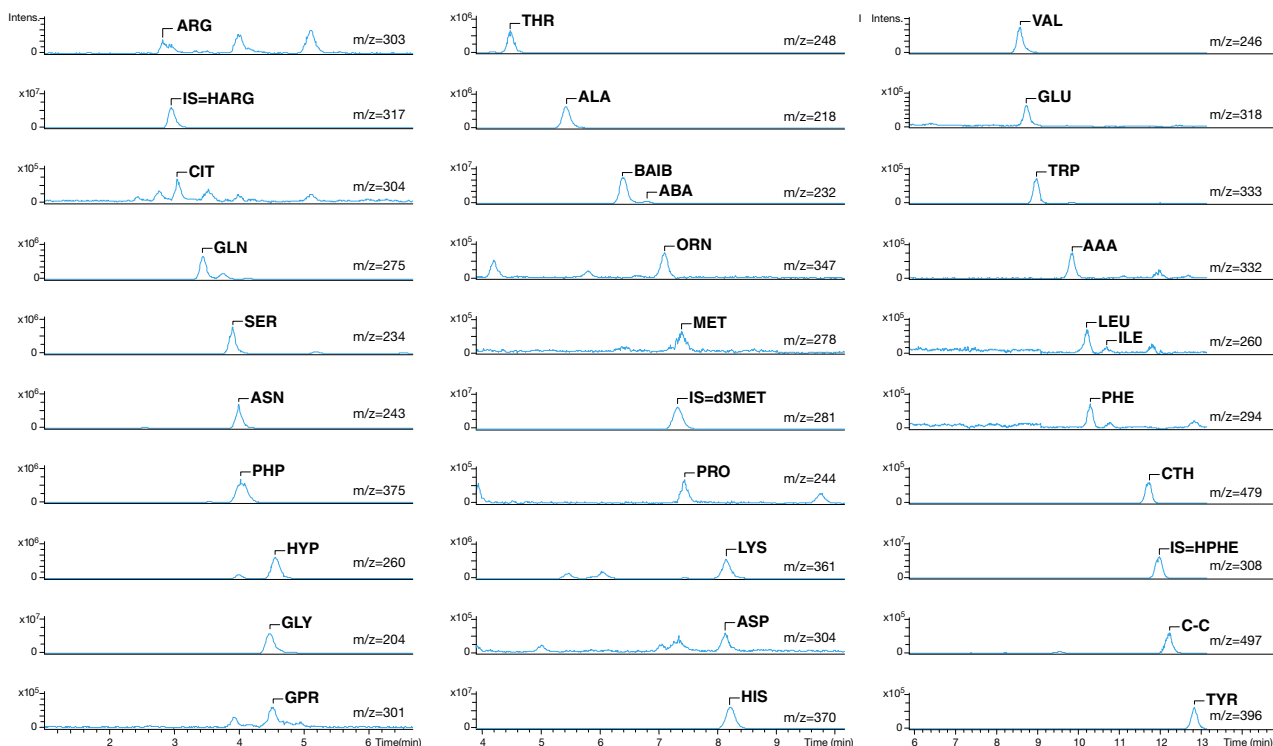
Amino Acids Sample Mix

Kit: EZ:faast LC/MS for Free (Physiological) Amino Acids
Order No.: KH0-7338
HPLC Column: EZ:faast 4µ AAA-MS 250 x 3.0mm
Mobile Phase: A: 10mM ammonium formate in water
 B: 10mM ammonium formate in methanol
 68 to 92%B in 12min, re-equilibration at 68%B for 4min
Flow Rate: 0.5 mL/min
Injection Volume: 5µL
Detection: Bruker Esquire 2000 IT ESI
Scan Range: 100-600 m/z
Sample: Derivatized amino acids in human urine (0.1mL). Internal standards (HARG, d3MET and HPHE) were added at a concentration of 200 µmol/L each.

EZ:Faast™ AMINO ACID ANALYSIS

LC/MS Clinical Applications

Amino Acids in Human Urine



App ID 14770

Amino Acids in Human Urine

Kit: EZ:faast LC/MS for Free (Physiological) Amino Acids
Order No.: KH0-7338
HPLC Column: EZ:faast 4µ AAA-MS 250 x 3.0mm
Mobile Phase: A: 10mM ammonium formate in water
 B: 10mM ammonium formate in methanol
Gradient: 68 to 92%B in 12min, re-equilibration at 68%B for 4min
Flow Rate: 0.5 mL/min
Injection Volume: 5µL
Detection: Bruker Esquire 2000 IT ESI
Scan Range: 100-600 m/z
Sample: Derivatized amino acids in human urine (0.1mL). Internal standards (HARG, d3MET and HPHE) were added at a concentration of 200 µmol/L each.

- | | | |
|---------------------------|----------------------------|----------------------------|
| 1. Arginine | 12. Alanine | 23. Glutamic acid |
| 2. Homoarginine (IS) | 13. β-Aminoisobutyric acid | 24. Tryptophan |
| 3. Citrulline | 14. α-Aminobutyric acid | 25. α-Amino adipic acid |
| 4. Glutamine | 15. Ornithine | 26. Leucine |
| 5. Serine | 16. Methionine | 27. Isoleucine |
| 6. Asparagine | 17. d3-Methionine (IS) | 28. Phenylalanine |
| 7. Proline-hydroxyproline | 18. Proline | 29. Cystathionine |
| 8. 4-Hydroxyproline | 19. Lysine | 30. Homophenylalanine (IS) |
| 9. Glycine | 20. Aspartic acid | 31. Cystine |
| 10. Glycine-proline | 21. Histidine | 32. Tyrosine |
| 11. Threonine | 22. Valine | |

Select amino acids analyzed by LC/MS in Urine

Amino Acid	Detection Limit (nmol/mL)	RSD (n=30)%
Arginine	0.2	<4.9
Ornithine	0.05	<2.7
Methionine	0.05	<2.7
Valine	0.08	<4.9
Phenylalanine	0.1	<3.4
Cystathionine	0.02	<3.9
Cystine	0.02	<4.6

AMINO ACID ANALYSIS

EZ:faast

EZ:Faast™ AMINO ACID ANALYSIS

Amino Acid Composition Analysis of Proteins

- Internal standard throughout sample preparation for improved quantitation
- Compatible with most hydrolysis methods
- Post-hydrolysis acid dry-down steps not required
- SPE cleanup removes hydrolysis generated contaminants

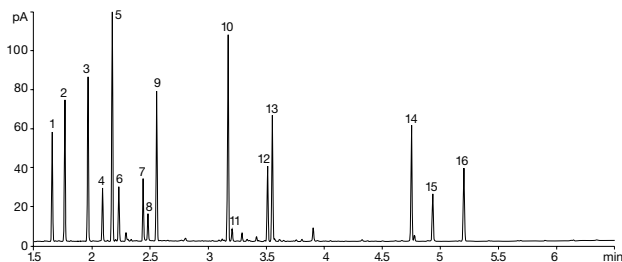
Traditionally amino acid composition analysis of proteins is performed after hydrolyzing the protein of interest using either liquid phase or vapor phase hydrolysis. EZ:faast hydrolysis kit is compatible with most common hydrolysis methods. Internal standards throughout the entire sample preparation process provide improved quantitation of hydrolyzed proteins. The SPE cleanup provided with EZ:faast removes any contamination due to hydrolyzed glycosylation products, and eliminates long acid dry-down steps. Hydrolyzed samples are quickly neutralized and ready for rapid EZ:faast analysis!

App ID 14892

Amino Acid Composition of a Standard Protein

Kit: EZ:faast GC/FID Protein Hydrolysate Kit
Order No.: KG0-7167
Injection: Split 1:15 @ 250°C, 2.0µL
Carrier Gas: Helium 1.5mL/minute, constant flow @ 110°C
Oven Program: 30°C/min from 110° to 320°C, hold at 320° for 1 minute
Detector: FID @ 320°C
Sample: Acid Hydrolyzed Carbonic Anhydrase: Sample was hydrolyzed using vapor phase acid hydrolysis; 6N HCl with 1% phenol for 24 hours at 110°C. Sample is neutralized and analyzed directly by EZ:faast.

1. Alanine	9. Proline
2. Glycine	10. Aspartic Acid
3. Valine	11. Methionine
4. Norvaline (IS)	12. Glutamic Acid
5. Leucine	13. Phenylalanine
6. Isoleucine	14. Lysine
7. Threonine	15. Histidine
8. Serine	16. Tyrosine



Composition Results for a Standard Protein

Sample Name: Bovine Carbonic Anhydrase II
Volume Sampled (µL): 20
Expected Concentration (mg/mL): 4.500

Amino Acids	Observed Amount (nmoles)	Theoretical No. of Residues	Observed No. of Residues	Amino Acid % Error
ALA	54.4	17	17.3	1.7
GLY	77.4	20	24.6	23.0
VAL	69.6	20	22.1	10.6
NVAL(IS)	20	20	N/A	N/A
LEU	80.5	26	25.6	1.6
ILE	24.8	5	7.9	57.6
THR	42.5	14	13.5	3.6
SER	27.8	16	8.8	44.8
PRO	53.5	19	17.0	10.6
ASX	95.5	29	30.3	4.6
MET	4.8	3	1.5	49.2
GLX	71.1	22	22.6	2.7
PHE	32.5	11	10.3	6.1
LYS	67.2	18	21.4	18.6
HIS	30.3	11	9.6	12.5
TYR	20.3	8	6.5	19.4
CYS	Not Analyzed	0	Not Analyzed	
TRP	Not Analyzed	7	Not Analyzed	
ARG	Not Analyzed	9	Not Analyzed	

Expected Concentration (mg/mL)	Protein Hydrolyzed (nmoles)	Observed Concentration (mg/mL)	Int. Std. Corrected Concentration (mg/mL)	Sum of Average % Error
4.500	3.15	4.49	4.49	17.8

Protein Molecular Weight	Total µg Hydrolyzed	Correlation Coefficient
28533	89.80	0.989



AMINO ACID ANALYSIS

EZ:faast

EZ:Faast™ AMINO ACID ANALYSIS

App ID 14344

Amino Acids in Potato Tissue

Kit: EZ:faast GC/FID
Free (Physiological) Amino Acid Kit
KGO-7165

Order No.: KGO-7165

Injection: Split 1:15 @ 250°C, 2.5 µL

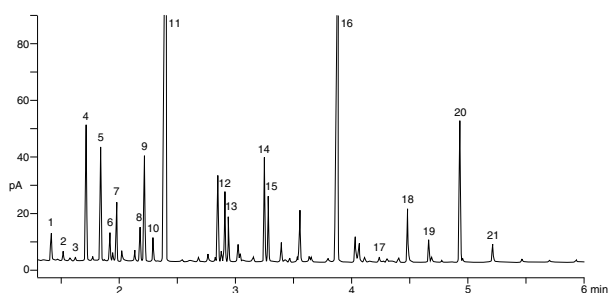
Carrier Gas: Helium 1.5 mL/minute, constant flow @ 110°C

Oven Program: 30°C/min from 110° to 320°C, hold at 320° for 1 minute

Detector: FID @ 320°C

Sample: Derivatized amino acids in potato tissue (0.1 mL). Norvaline is the internal standard added at a concentration of 200 µmol/L

- Alanine
- Glycine
- α-Aminobutyric acid
- Valine
- Norvaline (IS)
- Leucine
- Isoleucine
- Threonine
- Serine
- Proline
- Asparagine
- Aspartic Acid
- Methionine
- Glutamic Acid
- Phenylalanine
- Glutamine
- Ornithine
- Histidine
- Tyrosine
- Tryptophan



App ID 14607

Amino Acids in Fermentation Broth

Kit: EZ:faast GC/FID
Free (Physiological) Amino Acid Kit
KGO-7165

Order No.: KGO-7165

Injection: Split 1:15 @ 250°C, 2.5 µL

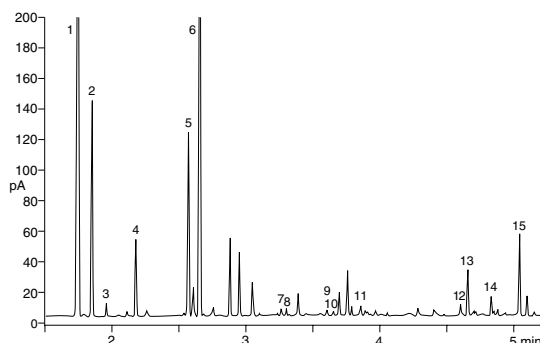
Carrier Gas: Helium 1.5 mL/minute, constant flow @ 110°C

Oven Program: 30°C/min from 110° to 320°C, hold at 320° for 1 minute

Detector: FID @ 320°C

Sample: Derivatized amino acids in fermentation broth (0.1 mL). Norvaline is the internal standard added at a concentration of 200 µmol/L

- Alanine
- Glycine
- α-Aminobutyric acid
- Norvaline (IS)
- Serine
- Proline
- Asparagine
- Methionine
- Glutamic Acid
- Phenylalanine
- α-Amino adipic Acid
- Ornithine
- Glycine-proline (dipeptide)
- Lysine
- Histidine



Technical Specifications

Detection Limit:	1 nmol/mL by GC/FID, better by MS
Precision in Quantitation, %RSD:	+/-15% (<10% for most)
Reproducibility of Retention Time, %RSD:	<1%
Samples Analyzed per Kit:	384 samples
Chromatographic System:	GC/FID, GC/NPD, GC/MS or LC/MS system
Chromatographic Column:	Zebtron GC column of proprietary phases or AAA LC column



Evaluate EZ:Faast™ in your lab for 45 days, if you are not completely satisfied return it for a full refund.

ORDERING INFORMATION

Amino Acid Analysis Kits

Each kit includes: ZB-AAA GC column, or AAA LC column, sample prep and derivatization reagents, sample prep vials, AA standards, SPE pipette tips, vial rack, autosampler vials with inserts with MS kits and microdispenser.

Order No.	Description	Unit	Price
KGO-7165	GC/FID Free (Physiological) Amino Acid Analysis Kit	ea	
KGO-7166	GC/MS Free (Physiological) Amino Acid Analysis Kit	ea	
KGO-7167	GC/FID Protein Hydrolysate Kit	ea	
KGO-7168	GC/MS Protein Hydrolysate Kit	ea	
KHO-7337	LC/MS Free (Physiological) Amino Acids Kit with 250 x 2.0mm column	ea	
KHO-7338	LC/MS Free (Physiological) Amino Acids Kit with 250 x 3.0mm column	ea	
KHO-7339	LC/MS Protein Hydrolysates Kit with 250 x 2.0mm column	ea	
KHO-7340	LC/MS Protein Hydrolysates Kit with 250 x 3.0mm column	ea	
AGO-7184	GC Free (Physiological) Amino Acid Standards (SD1, 2, 3) 2mL/vial x 2	ea	
AGO-7263	GC Protein Hydrolysate Standard (SD) 2mL/vial x 2	ea	
ALO-7500	LC/MS Free (Physiological) Amino Acid Standards (SD1, 2, 3) 2mL/vial x 2	ea	



AMINO ACID ANALYSIS

EZ:faast

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