

EZ:faast™ Amino Acid Analysis

U.S. Patent No. 6, 770, 246

Amino Acid Analysis in 15 Minutes

- Easy-to-use kit contains everything needed for sample clean up, derivatization, and analysis of 384 samples
- Kits compatible with GC/FID, GC/MS, & LC/MS chromatographic systems
- Simple & fast sample prep, derivatization, analysis for both physiological (free) amino acids and protein hydrolysates
- Analyze amino acids from the most complex matrixes such as blood, plasma, foodstuffs, and fermentation broth
- No plasma protein or urea removal required for physiological samples
- Great sample purity and improved reproducibility
- Method can be optimized for sulfur-containing and aromatic amino acids, along with numerous other amino acid derivatives

Performance Characteristics

Precision

EZ:faast allows for the quantitation of amino acids in various sample matrixes with good precision. RSD values are listed for most applications shown in this section of the catalog. These values are better than 5 % for most amino acids and sample types, except for ASN, GLN, HIS, ILE, MET, TRP and TYR with % RSD<12. RSD % values include variation due to both sample preparation and analysis, other methods usually only consider analysis.

Accuracy

Recovery of EZ:faast varies between 89-107 %. An example of % recovery for spiked amino acids in specific matrixes is shown below. Clover honey was used to demonstrate accuracy. Five samples were taken from a well-homogenized honey and tested for % recovery (accuracy), each sample was spiked with 200 nmols/ mL of five different amino acids. All data reported in nmol/mL.

	Standard Added	Honey + Standard	Standard Recovered	% Recovery (Accuracy)
ALA	199	280.11	201.74	101.4
ASN	209	360.48	187.42	89.7
PHE	197	248.46	185.76	94.3
LYS	188	239.50	180.05	95.8
TYR	200	208.11	182.33	91.2

Five spiked samples showed good recovery (89.7 - 101.4 %) for all amino acids analyzed. For another detailed accuracy example, please refer to Technical Note (TN-8001) – *Asparagine Analysis in Food Products*.

Sensitivity

Limits of detection (LOD) are shown for each analytical technique (GC/FID, GC/MS and LC/MS) in the lists of amino acids amenable for analysis by EZ:faast. LODs were determined for the amino acids included in the standard mixtures provided with the kit for calibration purposes.



Effective Sample Clean Up

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 matrixes such as plasma and urine.

Table 1: Common Protein Removal Protocols vs. EZ:faast

Comparative data showing amino acid concentrations in µmol/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)

Derivative Stability

EZ:faast amino acid derivatives are stable at room temperature for more than 24 hours. Samples prepared during the day can be left on the autosampler tray at room temperature for analysis during the night or the next day. Prepared samples can be stored for a couple of days refrigerated or frozen for longer periods of time. The stability of amino acid derivatives prepared with standard solutions passed through the EZ:faast sample preparation procedure was tested at different moments in time over a 19 hour period starting shortly after sample preparation. The first injection was used for instrument calibration. Average % RSD for 22 amino acids was 2.9. Glycine and Isoleucine showed minimal variation in response with 0.69 and 0.7 % RSD, respectively. HYP with RSD 11.07 % is the least stable amino acid derivative.

Robustness

Method of analysis for amino acids based on the EZ:faast procedure is robust. Results are unaffected by most deviations from the preferred sample preparation and analysis protocol. The following parameters have been evaluated for their effect on method robustness: GC instrument settings, maximum loading capacity of sorbent tips, sample pH, sample loading speed during SPE clean up, and reaction times allowed for derivatization. The only critical parameters to monitor are speed of sample load during the SPE step and minimum reaction time allowed for derivatization (total 3 min).

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Analyze Over 60 Amino Acids with Several Kit Options

Based on Sample Type

Free (Physiological) Amino Acids

The EZ:faast kits for free amino acids provide rapid clean up, derivatization, and analysis of amino acids from complex mixtures, while yielding a full amino acid profile in 15 minutes. The sample preparation cleans up amino acids from complex matrixes like blood, urine, cheese, cell cultures, and wine. Samples are pre-column derivatized to form stable amino acid derivatives.

Protein Hydrolysates

Within 15 minutes, hydrolyzed protein or peptide samples are prepared and derivatized for rapid sequencing. Sample preparation ensures non-hydrolyzed protein removal for more accurate analysis. Derivatized samples will not degrade rapidly.

Based on Chromatographic Instrument

Gas Chromatography (GC)

The GC kit options are compatible with a NPD, FID, or MS detectors. The analysis time on the GC column is a quick 8 minutes and provides excellent resolution of all amino acids in the profile. The derivatization procedure used makes the amino acids less polar and therefore more volatile so they can be analyzed via GC.

Liquid Chromatography (HPLC)

LC/MS kit options are available for labs without GC systems or to analyze Arginine or Citrulline. The sample preparation and derivatization is still just 7 minutes, but the analysis is slightly longer at 12 minutes.

Amino Acids Analyzed by GC

Chemical Name	Abbreviation	LOD* (nmol/mL) S/N 3:1	
		FID	MS
Alanine	ALA	1	0.1
β-Alanine	β-ALA		
Alliin			
α-Aminoadipic acid	AAA	1	0.2
4-Aminobenzoic acid	PABA		
α-Aminobutyric acid	ABA	1	0.2
β-Aminoisobutyric acid	β-AIB	4	0.2
β-Amino-n-butyric acid	βABA		
γ-Amino-n-butyric acid	GABA		
α-Aminopimelic acid	APA	0.47	0.4
Arginine-succinic acid	ARG-SUC		
Asparagine	ASN	2	2.5
Aspartame			
Aspartic acid	ASP	0.87	0.1
Bicin			

* LODs were determined for amino acids included in standard mixtures provided with the kit

Note: several amino acids coelute under the chromatographic conditions specified in the user manual

Amino Acids Analyzed by GC (cont'd)

Chemical Name	Abbreviation	LOD* (nmol/mL) S/N 3:1	
		FID	MS
Carboxymethyl-cysteine			
Chloro-phenylalanine	Cl-PHE		
Cystathionine	CTH	4	10
Cysteine	CYS		
Cystine	C-C	4	10
2,4-Diamino-n-butyric acid	DABA		
Diaminopimelic	DAPA		
3,4-Dihydroxyphenylalanine	DOPA		
Dopamine	DA		
Ethanolamine			
Ethionine	ETH		
Fluoro-alanine			
Glutamic acid	GLU	2	0.2
Glutamine	GLN	8	10
Glycine	GLY	2	0.1
Glycine-glycine (dipeptide)	GLY-GLY		
Glycine-proline (dipeptide)	GPR	1	5
Histamine	HA		
Histidine	HIS	1	0.2
Homocysteine	HCYS		
Homocystine	HC-CH		
Homophenylalanine	HPHE		
Homoserine	HSER		
Hydroxylysine (2 isomers)	HLV	2	10
3-Hydroxyproline	3HYP		
4-Hydroxyproline	4HYP	2	0.2
Isoleucine	ILE	0.65	0.2
allo-Isoleucine	alILE	0.65	0.1
Leucine	LEU	0.65	0.1
Lysine	LYS	1	0.2
Lysine-alanine (dipeptide)	LYS-ALA		
Methionine	MET	0.87	0.2
Methionine Sulfone			
Methionine Sulfoxide			
3-Methyl-cysteine			
Naphthyl-alanine			
3-Nitrotyrosine			
Norleucine	NLE		
Norvaline	NORV		
Ornithine	ORN	1	0.2
Phenylalanine	PHE	0.47	0.2
Phenyl Glycine	PHE-GLY		
Pipecolic Acid	HPRO		
Proline	PRO	1	0.1
Proline-hydroxyproline (dipeptide)	PHP	0.87	10
Sarcosine	SAR	1	0.1
Seleno Cystine	Se-C-C		
Seleno Methionine	Se-MET		
Serine	SER	2	0.2
Serotonin	SRO		
Theanine	THE		
Thioproline	TPR	0.43	0.1
Threonine	THR	2	0.2
Threonine-aspartic acid (dipeptide)	THR-ASP		
Tryptophan	TRP	0.43	0.1
Tyramine			
Tyrosine	TYR	0.4	0.2
Valine	VAL	0.6	0.2

EZ:faast™ Amino Acid Analysis

guarantee

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Medical and Clinical Toxicology

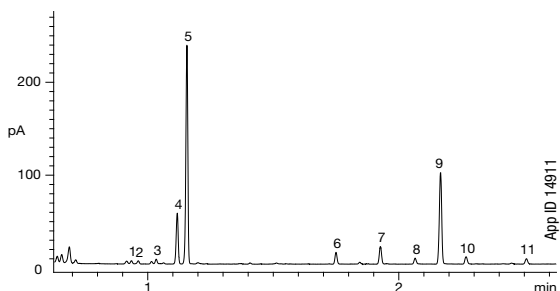
- Analyze complex blood, urine, and plasma samples

PKU (Phenylketonuria) Screening

Kit: EZ:faast GC/FID Free (Physiological) Amino Acid Kit
Part No.: KG0-7165
Injection: Split 1:15 @ 250 °C, 2 µL
Carrier Gas: Helium 2 mL/ minute constant flow @ 110 °C
Oven Program: 30 °C/ min from 110 °C to 320 °C, hold at 320 °C for 1 minute
Detector: FID @ 320 °C
Sample:

1. Aspartic Acid (10, 4.3)*	7. Lysine (52.5, 5.4)*
2. Methionine (12.5, 9.6)*	8. Histidine (20, 10.3)*
3. 4-Hydroxyproline (22.5, 3.5)*	9. Norvaline
4. Glutamic Acid (337.5, 2.8)*	10. Tyrosine (35, 11.1)*
5. Phenylalanine (755, 2.2)*	11. Tryptophan (7.5, 11.6)*
6. Ornithine (32.5, 3.6)*	

* Detection Limit (nmol/mL), RSD (n=30) %



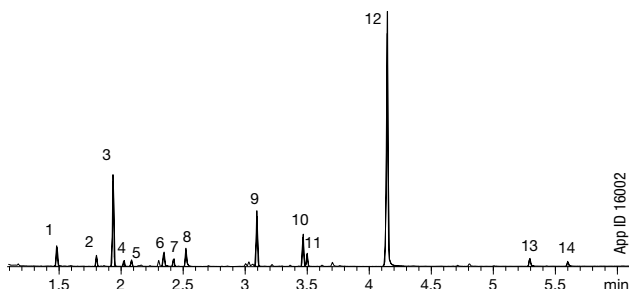
Food and Beverage

- Analyze complex wine, beer, juice, foodstuffs, fermentation broths, cell culture, and protein hydrolysate matrices

Theanine Analysis in Tea

Column: EZ:faast GC/MS Free (Physiological) Amino Acid Kit
Part No.: KG0-7166
Injection: Split 1:15 @ 250 °C, 2 µL
Carrier Gas: Helium 1.1 mL/ minute constant flow @ 110 °C
Oven Program: 32 °C/ min from 110 °C to 320 °C
Detector: MS @ 45-450 m/z
Sample:

1. Alanine	10. Glutamic Acid
2. Valine	11. Phenylalanine
3. Norvaline (IS)	12. Theanine & Glutamine
4. Leucine	13. Tyrosine
5. Isoleucine	14. Tryptophan
6. Serine	
7. Proline	
8. Asparagine	
9. Aspartic Acid	



Biotechnology and Pharmaceutical

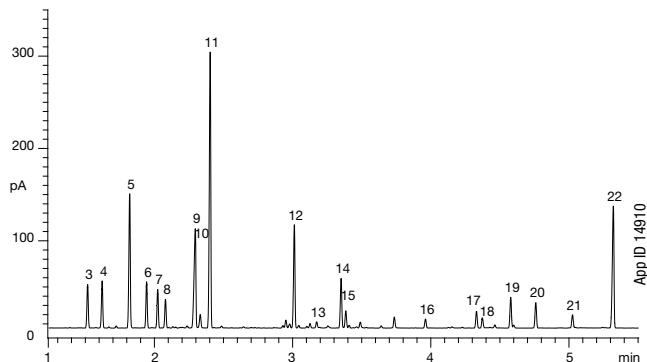
- For the analysis of amino acids in fermentation broths, cell cultures, serum, and protein hydrolysates

Mammalian Cell Culture

Kit: EZ:faast GC/FID Free (Physiological) Amino Acid Kit
Part No.: KG0-7165
Injection: Split 1:15 @ 250 °C, 2 µL
Carrier Gas: Helium 1.5 mL/ minute, constant flow @ 110 °C
Oven Program: 30 °C/ min from 110 °C to 320 °C, hold at 320 °C for 1 minute
Detector: FID @ 320 °C
Sample: Derivatized amino acids in mammalian cell culture (0.1 mL). Norvaline is the internal standard added at a concentration of 200 µmol/L.

3. Alanine (248, 2.1)*	13. 4-Hydroxyproline (45, 0.9)*
4. Glycine (304, 2.9)*	14. Glutamic Acid (538, 5.6)*
5. Valine (685, 3.8)*	15. Phenylalanine (50, 2.3)*
6. Norvaline (IS)	16. Glutamine (105, 10.5)*
7. Leucine (149, 2.2)*	17. Ornithine (93, 3.8)*
8. Isoleucine (138, 8.6)*	18. Glycine-proline (dipeptide) (57, 5.6)*
9. Threonine (891, 3.6)*	19. Lysine (134, 5.5)*
10. Serine (124, 4.8)*	20. Histidine (168, 10.5)*
11. Proline (1220, 2.3)*	21. Tyrosine (45, 9.2)*
12. Asparagine (630, 2.6)*	22. Tryptophan (462, 11.5)*

* Detection Limit (nmol/mL), RSD (n=30) %



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, and microdispenser for reagents 4 and 5. MS kits also include autosampler vials with inserts. GC kits also include injector liners.

Part No.	Description	Unit	Price
KG0-7165	GC/FID Free (Physiological) Amino Acid Analysis Kit	ea	
KG0-7166	GC/MS Free (Physiological) Amino Acid Analysis Kit	ea	
KG0-7167	GC/FID Protein Hydrolysate Kit	ea	
KG0-7168	GC/MS Protein Hydrolysate Kit	ea	
KH0-7337	LC/MS Free (Physiological) Amino Acids Kit with 250 x 2.0 mm column	ea	
KH0-7338	LC/MS Free (Physiological) Amino Acids Kit with 250 x 3.0 mm column	ea	
KH0-7339	LC/MS Protein Hydrolysates Kit with 250 x 2.0 mm column	ea	
KH0-7340	LC/MS Protein Hydrolysates Kit with 250 x 3.0 mm column	ea	
AG0-7184	GC Free (Physiological) Amino Acid Standards (SD1, 2, 3) 2 mL/vial x 2	ea	
AG0-7263	GC Protein Hydrolysate Standard (SD) 2 mL/vial x 2	ea	
AL0-7500	LC/MS Free (Physiological) Amino Acid Standards (SD1, 2, 3) 2 mL/vial x 2	ea	
AL0-7501	LC/MS Protein Hydrolysate Standard (SD) 2 mL/vial x 2	ea	