

**EZ:faast**<sup>™</sup>  
easy-fast amino acid sample testing kit  
Patent Pending

Can You Complete An Amino Acid Analysis  
In **15:00 Minutes?**  ...now you can!



Amino acid analysis kits for:

Sample Prep

Derivatization

GC or LC Analysis



**phenomenex**<sup>®</sup>   
...breaking with tradition<sup>sm</sup>

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# Amino Acid Sample Prep and Analysis in 15 Minutes

guarantee



Evaluate an EZ:faast kit in your lab for 30 days. If you are not completely satisfied with the performance, return it for a full refund.

**EZ:faast kits provide reagents and supplies for sample preparation, derivatization and GC/FID, GC/NPD, GC/MS and LC/MS analysis of over 50 amino acids and dipeptides in complex matrices and protein hydrolysates. Additionally the EZ:faast method offers:**

- **A standard 8 minute procedure for sample preparation and derivatization**
- **Elimination of labor-intensive protein and urea removal procedures**
- **Excellent method reproducibility (RSD <1%) and quantitation down to 1nmol/mL**

**Established methods for amino acid analysis in serum, urine, beer, wine, feeds, fermentation broths, foodstuffs and protein hydrolysates**

## **Obstacles in Current Amino Acid Protocols**

Ion-exchange chromatography using ninhydrin derivatization or reversed phase chromatography have traditionally been used on dedicated instruments for amino acid analysis. Current ion-exchange methods originated in the late 1940s and are known for lengthy 60-180 minute runtimes along with extensive sample preparation to remove interfering components (such as proteins or urea). Ion-exchange methods typically use expensive, dedicated instruments that are difficult to maintain and operate. Other methods, relying on reversed-phase HPLC, have been developed that can be run on any HPLC system saving the expense of purchasing and maintaining a dedicated amino acid analyzer. However, reversed phase HPLC methods can suffer from numerous deficiencies including: long analysis times, laborious sample preparation protocols, poor peak resolution, marginal quantification, and limited stability of derivatized amino acids. Laboratories needing a rapid and simple method to process high volumes of samples may find traditional amino acid analysis laborious and costly. EZ:faast Amino Acid analysis kits has been developed to overcome these obstacles with a simple kit containing reagents and supplies to perform sample preparation, derivatization and improved quantitation using any GC/FID, GC/NPD, or GC/MS system in 15 minutes or by LC/MS in 24 minutes.

## Economical, Hassle-Free Amino Acid Analysis

### Free (Physiological) Amino Acids

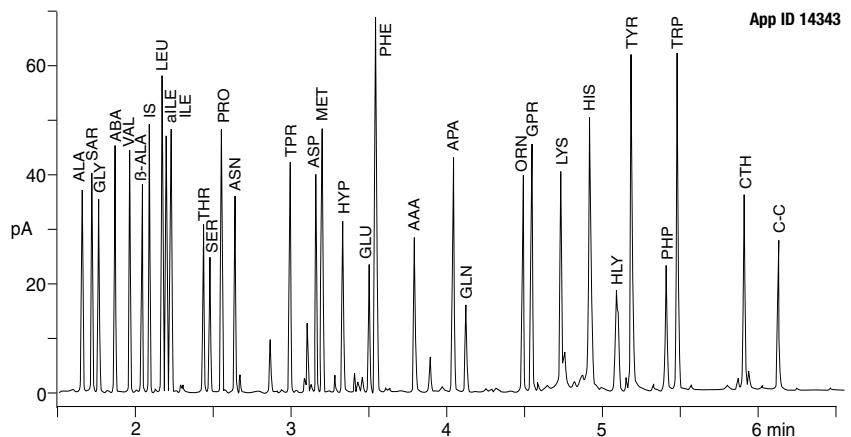
Free amino acids are routinely analyzed in physiological fluids, feeds, fermentation broths and other sources for basic research, nutritional labeling and more recently for patient diagnosis. Metabolic deficiencies are diagnosed based on the levels of particular amino acids in body fluids. While some protocols may provide adequate chromatographic methods and derivatization procedures, sample throughput remains slow due to lengthy sample cleanup and preparation requirements. Using the EZ:faast Free (Physiological) Amino Acid GC and LC/MS kits, sample preparation time is only 7 minutes regardless of sample matrix. Blood, plasma, urine, cerebral spinal fluid, wine or grain samples contain interfering proteins, urea and other impurities that can lead to poor chromatographic results. In 7 minutes, EZ:faast can remove interfering components leaving a highly pure and derivatized amino acid sample ready for injection. Analysis time is dramatically reduced and quantitation significantly improved over traditional methods; this makes for a rapid and sensitive method for any lab (Figure 1). For labs with a large number of samples, EZ:faast truly provides rapid throughput. Without the need for lengthy sample preparation, labor cost is dramatically decreased, making EZ:faast a much more economical method.

## Protein and Peptide Hydrolysates

Numerous labs perform quality control assays of both natural and synthetic proteins and peptides by quantifying the hydrolyzed amino acid composition of their final protein product. Protein core labs will find the EZ:faast kits excellent for confirming protein identity and purity quickly. Using an EZ:faast GC kit, results are obtainable in about 15 minutes and in 24 minutes when using the EZ:faast LC/MS kit. For labs analyzing large number of samples the short analysis time dramatically increases productivity and reduces the cost of sample analysis.

Proteins are long linear strings of amino acids connected to each other by peptide bonds. Hydrolysis of the peptide bonds releases the individual amino acids. The EZ:faast system allows rapid purification and derivatization of the released amino acids. Composition and quantity of hydrolyzed amino acids can then be determined quickly and accurately (Figure 2). The overall system provides both a rapid and simple sample preparation and analysis procedure, and is amenable to limited automation. More samples can now be analyzed over a given amount of time for less money as labor cost is dramatically decreased by this simple and rapid procedure.

**Figure 1**  
Free Amino Acid  
Standards Analyzed  
by EZ:Faast



**Figure 2**  
Protein Amino Acids  
Standards Analyzed  
by EZ:faast

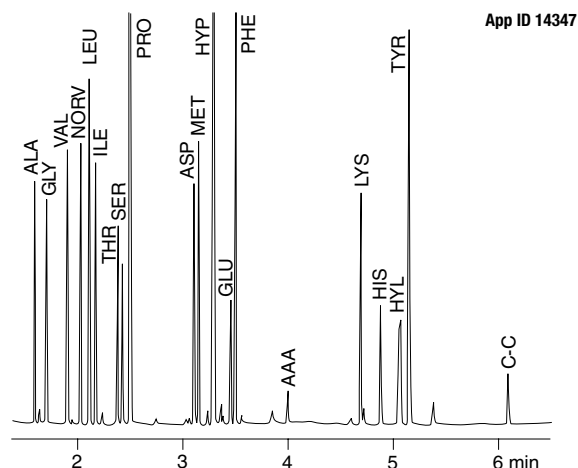


Table 1

**Analyze Over 50 Amino Acids**

| Abbreviation          | Alt. Abbreviation:  | Chemical Name                       | SIM           |
|-----------------------|---------------------|-------------------------------------|---------------|
| ALA                   | A                   | Alanine                             | 130, 70       |
| SAR                   | SA                  | Sarcosine                           | 130, 58       |
| GLY                   | G                   | Glycine                             | 116, 74       |
| ABA                   |                     | $\alpha$ -Aminobutyric acid         | 144, 102      |
| VAL                   | V                   | Valine                              | 158, 72       |
| $\beta$ -ALA          |                     | $\beta$ -Alanine                    | 217, 129, 116 |
| $\beta$ -AIB          | BAIBA               | $\beta$ -Aminoisobutyric acid       | 172, 143, 84  |
| $\beta$ -ABA          |                     | $\beta$ -Amino-n-butyric acid       | 216, 172, 144 |
| NORV                  |                     | Norvaline                           | 158, 72       |
| LEU                   | L                   | Leucine                             | 172, 86       |
| ILE                   | I                   | Isoleucine                          | 172, 130      |
| aILE                  |                     | Allo-Isoleucine                     | 172, 130      |
| HSER                  |                     | Homoserine                          | 101, 128, 143 |
| NLE                   |                     | Norleucine                          | 172, 86       |
| THR                   | T                   | Threonine                           | 160, 101      |
| SER                   | S                   | Serine                              | 146, 203      |
| PRO                   | P                   | Proline                             | 156, 243      |
| GABA                  |                     | $\gamma$ -Amino-n-butyric acid      | 130, 144, 17  |
| ASN                   | N                   | Asparagine                          | 155, 69       |
| TPR                   |                     | Thiaproline                         | 174, 147      |
| ASP                   | D                   | Aspartic acid                       | 216, 130      |
| MET                   | M                   | Methionine                          | 203, 277      |
| HYP                   | OHPro               | 4-Hydroxyproline                    | 172, 86       |
| GLU                   | E                   | Glutamic acid                       | 230, 170      |
| PHE                   | F                   | Phenylalanine                       | 206, 190      |
| AAA                   |                     | $\alpha$ -Amino adipic acid         | 244, 98       |
| CYS                   | C                   | Cysteine                            | 248, 162, 206 |
| PABA                  |                     | 4-Aminobenzoic acid                 | 265, 206, 163 |
| HCYS                  |                     | Homocysteine                        | 142, 203      |
| APA                   |                     | $\alpha$ -Aminopimelic acid         | 198, 258, 286 |
| HA                    |                     | Histamine                           | 180, 168, 94  |
| THE                   |                     | Theanine                            |               |
| MET-SO                |                     | Methionine Sulfoxide                |               |
| GLN                   | Q                   | Glutamine                           | 84, 187       |
| DABA                  |                     | 2,4-Diamino-n-butyric acid          | 203, 142, 245 |
| GLY-GLY               |                     | Glycine-glycine (dipeptide)         | 117, 144, 201 |
| MET-SO <sub>2</sub>   |                     | Methionine Sulfone                  |               |
| ORN                   | O                   | Ornithine                           | 156, 70       |
| GPR                   |                     | Glycine-proline (dipeptide)         | 70, 300       |
| LYS                   | K                   | Lysine                              | 170, 128      |
| THR-ASP               |                     | Threonine-aspartic acid (dipeptide) | 218, 360, 130 |
| HIS                   | H                   | Histidine                           | 282, 168      |
| Se-CYS                |                     | Selenocystine                       |               |
| HLY                   | OHLys               | Hydroxylysine (2 isomers)           | 129, 169      |
| TYR                   | Y                   | Tyrosine                            | 206, 107      |
| DAP                   |                     | Diaminopimelic acid                 | 256, 168, 196 |
| PHP                   |                     | Proline-hydroxyproline (dipeptide)  | 156, 114      |
| TRP                   | W                   | Tryptophan                          | 130           |
| NTYR                  |                     | 3-Nitrotyrosine                     |               |
| LYS-ALA               |                     | Lysine-alanine (dipeptide)          | 170, 224, 153 |
| DA                    |                     | Dopamine                            | 179, 136, 123 |
| CTH                   |                     | Cystathionine                       | 203, 272      |
| DOPA                  |                     | 3,4-Dihydroxyphenylalanine          | 222, 123      |
| C-C                   | (Cys) <sub>2</sub>  | Cystine                             | 248, 216      |
| HC-HC                 | (Hcys) <sub>2</sub> | Homocystine                         | 230, 188, 128 |
| ARG-SUC               |                     | Arginino-succinic acid              | 441, 326      |
| Et(OH)NH <sub>2</sub> |                     | Ethanolamine                        | 116, 117      |
| ETH                   |                     | Ethionine                           | 203, 291, 143 |
| PA                    |                     | Pipecolic acid                      |               |

**Additional Amino Acids analyzed by the EZ:faast LC/MS kit**

| Abbreviation | Alt. Abbreviation: | Chemical Name                    | SIM |
|--------------|--------------------|----------------------------------|-----|
| ARG          |                    | Arginine                         | 303 |
| CIT          |                    | Citrulline                       | 287 |
| SDMA         |                    | Dimethyl arginine (Symmetrical)  |     |
| ADMA         |                    | Dimethyl arginine (Asymmetrical) |     |
| 1MHIS        |                    | 1-Methyl-histidine               | 298 |
| 3MHIS        |                    | 3-Methyl-histidine               | 298 |

**50 Amino Acids & Related Compounds Quantified in 15 Minutes**

The EZ:faast method is currently designed to analyze over 50 amino acids and related compounds (Table 1). All peaks can be accurately and reproducibly quantified. Additional amino acids can be analyzed with little to no modification of the standard methodology. Please contact Phenomenex for additional amino acids analyzed using the EZ:faast method.

## EZ steps

Colors added for illustration purposes only.



STEP 1  
00:00:00

**Step 1.** 0 min  
Pipette sample and combine internal standard (yellow).  
**Advantage:** Internal standard for improved quantitation.



STEP 2  
00:01:00

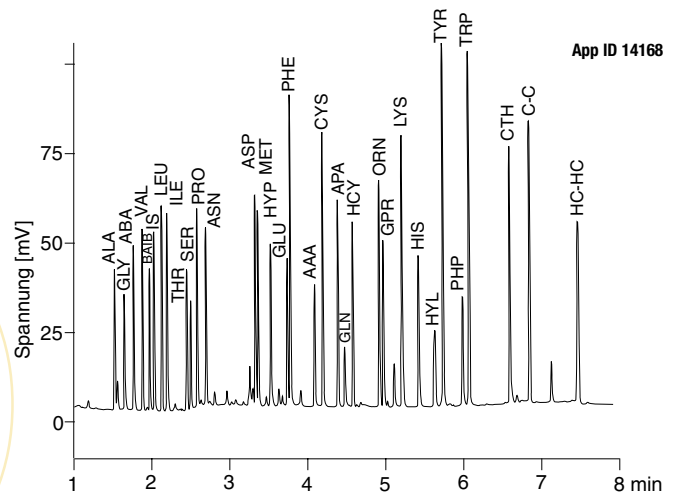
**Step 2.** 1 min  
Pipette sample through SPE sorbent tip.  
**Advantage:** No lengthy protein or urea removal.

## 8 Simple Steps



**Step 8.** 15 min  
Analyze the chromatogram  
**Advantage:** Full resolution of over 50 amino acids and related compounds in a 7 minute GC run.

\* 15 minute sample prep and analysis time applies to GC kits only. Sample prep and analysis time by LC/MS is 24 minutes.



**Step 7.** 7 min  
Sample preparation complete – inject onto GC/FID, GC/NPD, GC/MS or LC/MS system.  
**Advantage:** Very stable derivatized sample reduces losses due to degradation.



EZ  
steps

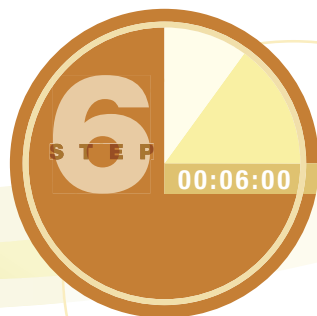
**Step 3.** 2 min  
Draw wash solution (blue)  
through SPE sorbent tip.  
**Advantage:** Outstanding  
sample purity for easy and  
accurate quantitation.



**Step 4.** 3 min  
Expel amino acids (blue)  
from tip with Eluting Medium  
(clear).  
**Advantage:** Almost no loss of  
amino acids.



**Step 5.** 4 min  
Add organic derivatizing reagent  
(orange).  
**Advantage:** Derivatization is  
complete in 2 min and can be  
done in aqueous phase.



**Step 6.** 6 min  
Extract amino acid derivatives  
from aqueous layer (light green)  
into organic layer (orange).  
**Advantage:** Additional  
purification step.

## in 15 Minutes!

The EZ:faast procedure's greatest benefit is the rapid sample preparation and derivatization method. For complex matrices like plasma, urine, fermentation broths or feeds this procedure can provide results equal to traditional protein and urea removal methods in less time. Even for relatively clean samples like protein hydrolysates, this method shows improvement in quantitation due to cleaner samples and more stable amino acid derivatives. Shorter sample preparation times cut labor time and cost, making this a highly cost effective assay for amino acid analysis (less than \$4 a sample). Below is a simplified diagram illustrating each of the major steps within the EZ:faast protocol.

## 8 minutes of Sample Prep

### No Protein or Urea Removal Required

The analysis of free amino acids in physiological fluids, grains, fermentation broths and other complex matrices differs from the analysis of protein hydrolysates due to the high concentration of interfering compounds. A relatively large amount of proteins, peptides, urea and other matrix components have to be removed prior to free amino acid analysis by GC or HPLC, otherwise columns deteriorate rapidly, quantitation is poor, and analysis results are not reproducible. Current procedures for de-proteinization and urea removal are labor intensive and recoveries are low for some amino acids. Also, reagents used for de-proteinization can interfere in the amino acid profile. The EZ:faast method, however, does not require traditional de-proteinization or urea removal methods to be followed; proteins are excluded from the sample as it passes through an SPE sorbent tip. The SPE sorbent binds amino acids while proteins and urea are washed away leaving only the free amino acids. A comparison of the EZ:faast method to other common de-proteinization methods shows as good or better results using the EZ:faast method.

EZ:faast translates into major cost and time savings. Hydrolyzed protein samples are often in a clean sample matrix. Hydrolysis under strong acidic conditions at elevated temperatures causes proteins and peptides to breakdown into their component amino acids. Other components like hydrolyzed carbohydrates and lipids, are excluded from the sample as it passes through the SPE sorbent tip. With purer samples and better derivative stability – quantitation and method reproducibility can be improved.

Table 2: Data representing a series of comparative tests on the same plasma sample with and without protein removal; analysis of all samples was performed using the EZ:faast kit. The data represents the calculated amino acid concentration in  $\mu\text{mol/L}$  after the sample has undergone three common de-proteinization procedures (SSA = sulfosalicylic acid; TCA = trichloroacetic acid; ORG = acetonitrile:ethanol 2:1) compared to the EZ:faast procedure. The comparative data (mean values for 12 measurements) is presented in the table below. Results for amino acid content show no significant difference between samples with or without protein removal.

**Table 2**

|                        | <b>Without<br/>De-proteinization</b><br>(Standard EZ:faast method) | <b>SSA</b><br>(Recommended for OPA<br>derivatized samples) | <b>TCA</b>      | <b>ORG</b><br>(Recommended for PITC<br>derivatized samples) |
|------------------------|--|--|-----------------|---|
| <b>Procedure Time:</b> | <b>7 minutes</b>   | <b>≥3 hours</b>  | <b>≥3 hours</b> | <b>≥3 hours</b>   |
| 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)  |
| GLU                    | 40 (38-41)   | 35 (25-44)   | 35 (29-40)      | 35 (30-39)  |
| THR                    | 176 (170-181)  | 166 (161-171)  | 153 (145-161)   | 177 (168-186)   |
| SER                    | 142 (136-147)  | 133 (129-136)  | 129 (121-137)   | 136 (122-150)   |
| HYP                    | 14 (13-14)   | 12 (11-12)   | 13 (12-13)      | —   |
| ASN                    | 34 (32-35)   | 34 (33-35)   | 31 (27-34)      | 31 (28-33)  |
| GLN                    | 540 (495-584)  | 588 (561-615)  | 553 (514-592)   | 569 (554-584)   |
| C-C                    | 41 (39-42)   | 41 (39-42)   | 45 (41-49)      | 30 (21-39)  |
| PHE                    | 58 (56-60)   | 58 (56-59)   | 63 (59-67)      | 60 (57-63)  |
| TYR                    | 62 (59-64)   | 62 (58-66)   | 63 (59-66)      | 60 (57-63)  |
| TRP                    | 48 (46-49)   | 44 (41-47)   | 45 (42-48)      | 50 (43-56)  |
| ORN                    | 60 (58-61)   | 68 (62-73)   | 64 (60-67)      | 46 (43-49)  |
| LYS                    | 179 (172-185)  | 200 (178-222)  | 184 (162-205)   | 118 (108-127)   |
| HIS                    | 84 (78-89)   | 89 (82-96)   | 90 (85-95)      | 82 (73-91)  |



### Derivatization Simplified

After the very short sample cleanup procedure, the amino acids are quickly derivatized at room temperature with the addition of two reagents. The proprietary reagents modify both the carboxyl and amino groups of the amino acids forming derivatives stable at room temperature for several hours and stable at 4°C for several days. With good derivative stability, amino acid quantitation is more accurate and reliable; almost no amino acid sample is lost in sample preparation or through sample degradation. Reagents and supplies for sample preparation allow for the analysis of 384 samples per kit. Reagents, when stored according to label directions, are guaranteed for up to 12 months.

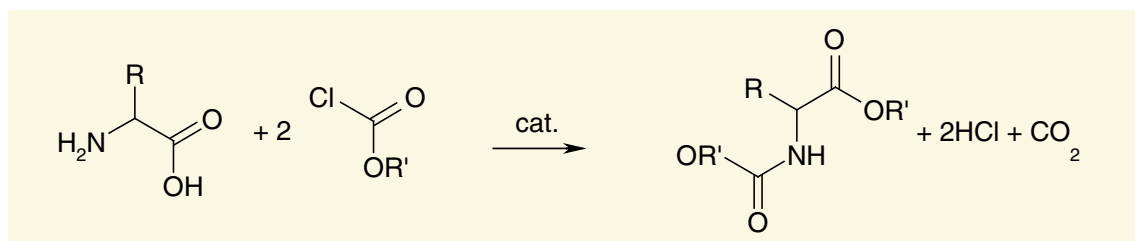
### Rapid Chromatographic Analysis

Traditional ion exchange and reversed phase methods for the analysis of amino acids utilize long 45-180 minute runtimes. The EZ:faast method takes advantage of the fast separation capability and high resolution of GC/FID, GC/NPD, GC/MS to analyze over 50 amino acids in about 8 minutes. The LC/MS chromatographic runtime is a

quick 12 minutes. High column efficiencies and resolution provides complete baseline separation for easy quantitation. Interfering compounds such as drug metabolites, proteins and urea that can be present in the sample matrix have been removed during sample preparation. With traditional methods, a large number of potential interfering compounds, like drugs and drug metabolites may co-migrate with amino acids thus affecting quantitation.

The high resolution GC method also allows for additional rare amino acids not listed in Table 1 to be analyzed without compromising throughput or resolution of other amino acids. In traditional HPLC based methods, rare amino acids may be analyzed but often results in a substantial increase of the analysis times – this is not the case with EZ:faast. The GC protocol allows for adjustments in carrier gas flow, oven temperature and the temperature gradient to ensure for the analysis of additional amino acids with a minor increase to the very short analysis time. High separation power and speed of the GC protocol also ensures method flexibility for the rapid analysis of additional amino acids with only slight (<5 min) increases in runtime.

**Figure 3** Simplified diagram illustrating EZ:faast derivatization reactions



accurate  
quantitation

EZ:faast kits provide reliable and accurate quantitation of over 50 fully baseline resolved amino acids. The lower limit of detection for EZ:faast is 1 nmol/mL and the precision in quantitation is <10% for most amino acids (a few amino acids show precisions in quantitation of +/- 15%). Sample loss and method precision is illustrated in the comparison of 5 injections of free amino acids extracted from plasma by a traditional ion exchange (IEX) method run on a dedicated amino acid analyzer and by the EZ:faast method (Table 3). EZ:faast provides improved quantitation due to several factors. Both sensitivity and reproducibility are improved with EZ:faast. Full baseline resolution accounts for some of the improvement, and the sample preparation procedure

insures nearly all interfering compounds are removed without sacrificing amino acid recovery. Once derivatized, amino acids are stable for several hours at room temperature and for several days if refrigerated, preventing sample loss by degradation.

Table 3. Comparative data for the analysis of plasma free amino acids by ion exchange chromatography (on a dedicated amino acid analyzer with ninhydrin detection), and by GC/FID (EZ:faast). Concentrations are given in nmol/mL (statistical evaluation by Horn).  $P_L$  stands for mean,  $L_L$  is lower limit;  $L_U$  is upper limit; IEX is ion exchange chromatography.

Table 3

|       | GLY   |          | ALA   |          | ABA  |          | VAL   |          | LEU   |          | ILE   |          | MET  |          | PRO   |          |
|-------|-------|----------|-------|----------|------|----------|-------|----------|-------|----------|-------|----------|------|----------|-------|----------|
|       | IEX   | EZ:faast | IEX   | EZ:faast | IEX  | EZ:faast | IEX   | EZ:faast | IEX   | EZ:faast | IEX   | EZ:faast | IEX  | EZ:faast | IEX   | EZ:faast |
| $P_L$ | 270.0 | 230.5    | 530.5 | 471.5    | 29.0 | 20.0     | 316.5 | 281.5    | 167.5 | 152.5    | 77.5  | 75.5     | 44.5 | 39.0     | 197.0 | 165.0    |
| $L_L$ | 265.8 | 194.9    | 515.8 | 406.6    | 24.8 | 7.4      | 272.5 | 254.3    | 119.3 | 104.3    | 54.5  | 44.1     | 38.2 | 30.6     | 37.9  | 160.8    |
| $L_U$ | 274.2 | 266.1    | 545.2 | 536.4    | 33.2 | 32.6     | 360.5 | 308.7    | 215.7 | 200.7    | 100.5 | 106.9    | 50.8 | 47.4     | 356.1 | 169.2    |
|       | 297   | 222      | 645   | 453      | 28   | 17       | 330   | 275      | 156   | 132      | 62    | 67       | 41   | 37       | 235   | 164      |
|       | 270   | 264      | 529   | 487      | 32   | 16       | 307   | 315      | 224   | 173      | 72    | 87       | 46   | 43       | 241   | 186      |
|       | 271   | 218      | 534   | 456      | 25   | 23       | 327   | 288      | 159   | 164      | 81    | 83       | 47   | 39       | 159   | 166      |
|       | 257   | 239      | 511   | 505      | 30   | 19       | 288   | 280      | 151   | 141      | 83    | 70       | 43   | 41       | 151   | 164      |
|       | 269   | 235      | 527   | 468      | 30   | 23       | 306   | 270      | 179   | 143      | 90    | 68       | 44   | 31       | 228   | 165      |

|       | THR   |          | SER   |          | ASP  |          | GLU   |          | GLN   |          | C-C  |          | PHE  |          | TYR   |          |
|-------|-------|----------|-------|----------|------|----------|-------|----------|-------|----------|------|----------|------|----------|-------|----------|
|       | IEX   | EZ:faast | IEX   | EZ:faast | IEX  | EZ:faast | IEX   | EZ:faast | IEX   | EZ:faast | IEX  | EZ:faast | IEX  | EZ:faast | IEX   | EZ:faast |
| $P_L$ | 175.0 | 163.5    | 117.5 | 91.0     | 33.5 | 17.0     | 147.5 | 142.0    | 622.5 | 575.0    | 28.0 | 23.5     | 76.5 | 74.0     | 82    | 97.5     |
| $L_L$ | 145.7 | 136.3    | 94.5  | 70.1     | 10.5 | 4.4      | 91.0  | 116.9    | 566.0 | 453.5    | 23.8 | 17.2     | 61.8 | 69.8     | 104.0 | 82.8     |
| $L_U$ | 204.3 | 190.7    | 140.5 | 111.9    | 56.5 | 29.6     | 204.0 | 167.1    | 679.0 | 696.5    | 32.2 | 29.8     | 91.2 | 78.2     | 91.4  | 112.2    |
|       | 209   | 146      | 123   | 81       | 43   | 14       | 114   | 136      | 739   | 493      | 44   | 23       | 84   | 83       | 82    | 94       |
|       | 179   | 165      | 119   | 107      | 35   | 14       | 163   | 128      | 636   | 563      | 29   | 25       | 73   | 74       | 107   | 101      |
|       | 182   | 170      | 123   | 96       | 39   | 24       | 161   | 148      | 636   | 604      | 21   | 31       | 76   | 73       | 106   | 107      |
|       | 168   | 157      | 112   | 86       | 28   | 18       | 152   | 137      | 609   | 546      | 27   | 22       | 80   | 66       | 108   | 91       |
|       | 159   | 179      | 99    | 94       | 19   | 20       | 134   | 157      | 596   | 624      | 27   | 20       | 66   | 75       | 101   | 96       |

|       | TRP |          | ORN  |          | LYS   |          | HIS   |          |
|-------|-----|----------|------|----------|-------|----------|-------|----------|
|       | IEX | EZ:faast | IEX  | EZ:faast | IEX   | EZ:faast | IEX   | EZ:faast |
| $P_L$ | -   | 57.5     | 87.0 | 58.0     | 185.0 | 153.5    | 118.0 | 106.0    |
| $L_L$ | -   | 34.5     | 74.4 | 37.1     | 159.0 | 130.5    | 270.0 | 59.9     |
| $L_U$ | -   | 80.5     | 99.6 | 78.9     | 210.0 | 176.5    | 270.0 | 152.1    |
|       |     | 63       | 81   | 53       | 198.0 | 159      | 114   | 92       |
|       |     | 52       | 89   | 78       | 182.0 | 167      | 122   | 118      |
|       |     | 57       | 100  | 57       | 191.0 | 158      | 127   | 112      |
|       |     | 67       | 84   | 51       | 170.0 | 144      | 114   | 95       |
|       |     | 41       | 90   | 63       | 179.0 | 148      | 115   | 117      |

## Accuracy and Precision

|  |   |
|--|---|
| Detection Limit                          | 1nmol/mL by GC/FID<br>1nmol/mL by GC/MS |
| Precision in Quantitation, %RSD          | +/-15% (<10% for most amino acids)      |
| Reproducibility of Retention Time, % RSD | <1%                                     |

**Sample:**

Derivatized amino acids in human serum (0.1mL),  
Norvaline is the internal standard added at a concentration of 200 µmol/L

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

**Free Amino Acids in Human Serum by GC/FID**

**Kit:** EZ:faast GC/FID Free (Physiological) Amino Acid Kit

**Order No.:** KG0-7165

**Injection:** Split 1:15 @ 250°C, 2.5µL

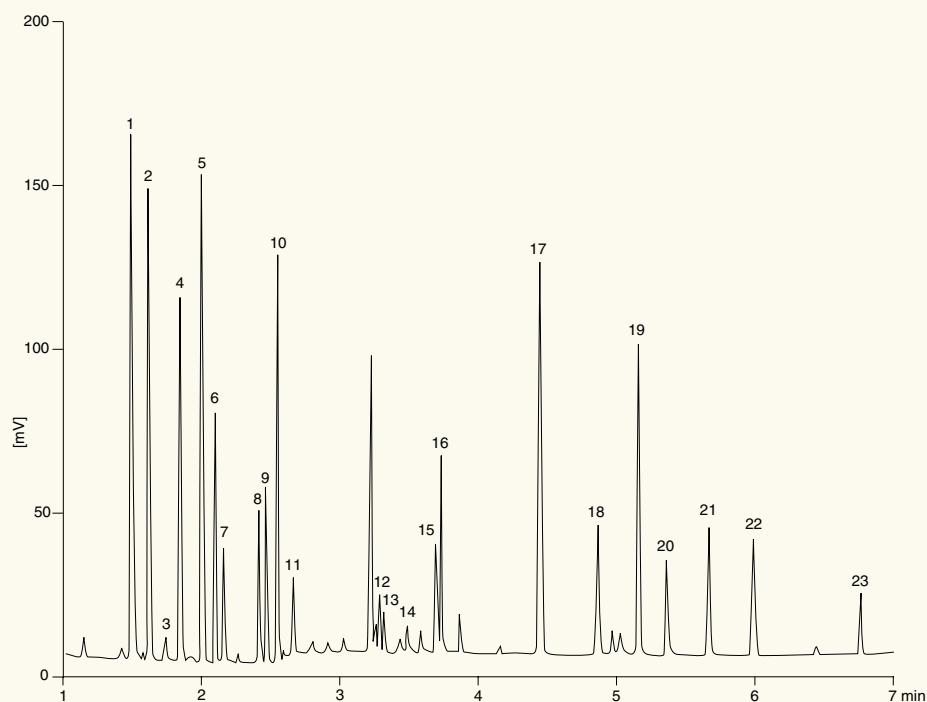
**Carrier Gas:** Helium 1.5mL/minute (60 kPa) @ 110°C

**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

App ID 14169



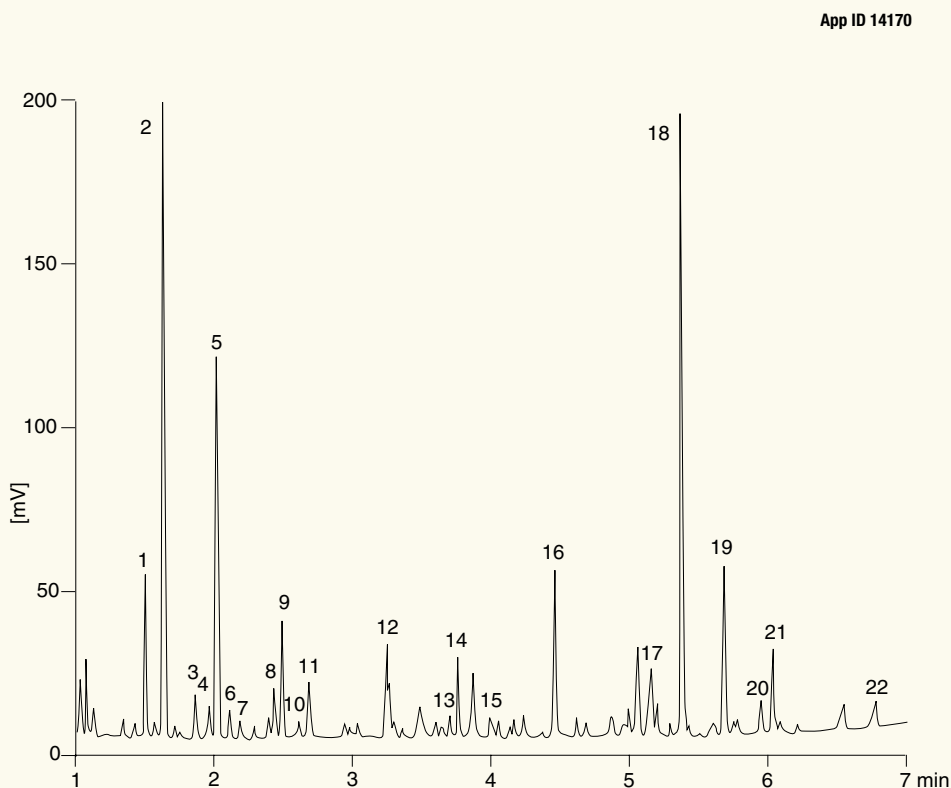
## Free Amino Acids in Human Urine by GC/FID

### Sample:

Derivatized amino acids in human urine (0.1 mL).

Norvaline is the internal standard added at a concentration of 200 µmol/L

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



**Kit:** EZ:faast GC/FID Free (Physiological) Amino Acid Kit

**Order No.:** KG0-7165

**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 potato tissue (0.1mL).

Norvaline is the internal standard added at a concentration of 200 µmol/L

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

**Free Amino Acids in Potato Tissue by GC/FID**

**Kit:** EZ:faast GC/FID Free (Physiological) Amino Acid Kit

**Order No.:** KGO-7165

**Injection:** Split 1:15 @ 250°C, 2.5µL

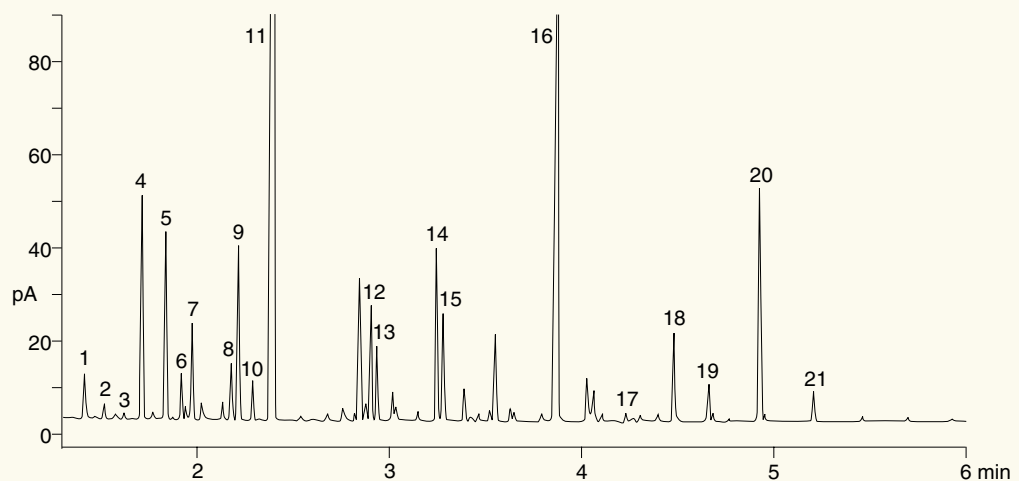
**Carrier Gas:** Helium 1.5mL/minute (60 kPa) @ 110°C

**Pressure Rise:** Constant pressure

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

**Detector:** FID @ 320°C

App ID 14344



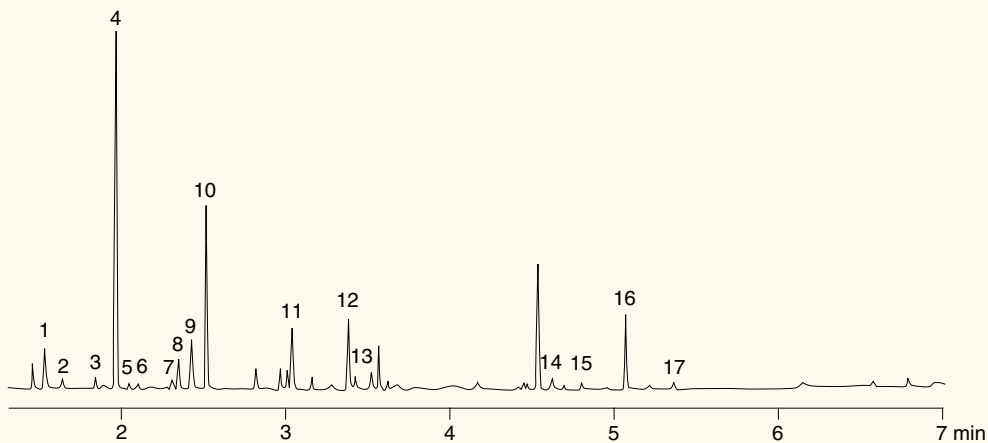
## Free Amino Acids in Corn Meal by GC/FID

### Sample:

Derivatized amino acids in corn meal (0.1mL).

Norvaline is the internal standard added at 200 µmol/L

- |                   |                   |
|-------------------|-------------------|
| 1. Alanine        | 11. Aspartic Acid |
| 2. Glycine        | 12. Glutamic Acid |
| 3. Valine         | 13. Phenylalanine |
| 4. Norvaline (IS) | 14. Lysine        |
| 5. Leucine        | 15. Histidine     |
| 6. Isoleucine     | 16. Tyrosine      |
| 7. Threonine      | 17. Tryptophan    |
| 8. Serine         |                   |
| 9. Proline        |                   |
| 10. Asparagine    |                   |



**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 8psi (60 kPa)

**Pressure Rise:** Constant pressure

**Oven Program:** 32°C/min from 110° to 320°C, hold at 320°C for 1 minute

**Detector:** FID @ 320°C

**Sample:**

Derivatized amino acids in wine (0.1mL).

Norvaline is the internal standard added at a concentration of 200 µmol/L

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

**Free Amino Acids in Wine by GC/MS**

**Kit:** EZ:faast GC/MS Free (Physiological) Amino Acid Kit

**Order No.:** KGO-7166

**Injection:** Split 1:15 @ 250°C, 2.5µL

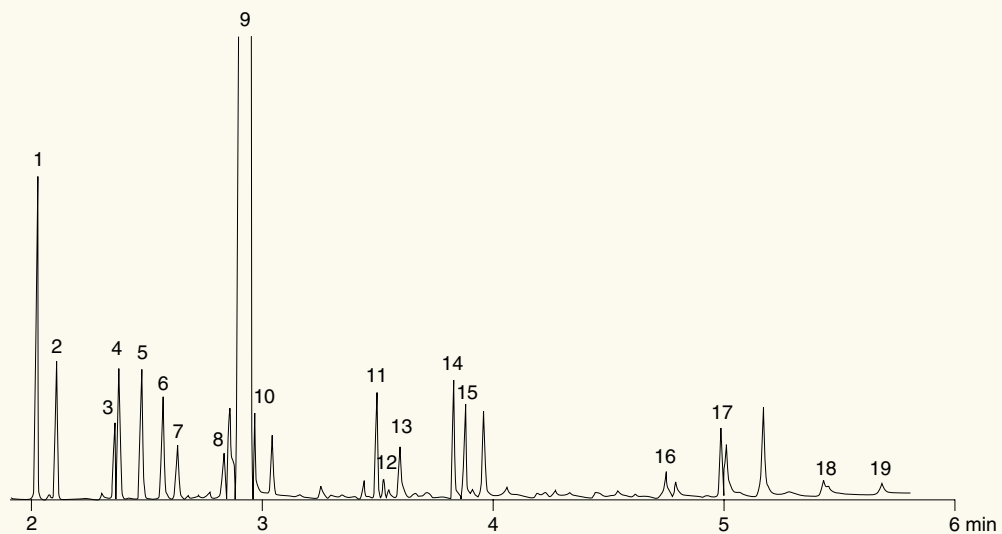
**Carrier Gas:** Helium 1.1mL/minute@110°C

**Pressure Rise:** 6 kPa/min

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

**Detector:** MS @ 300°C

App ID 14610



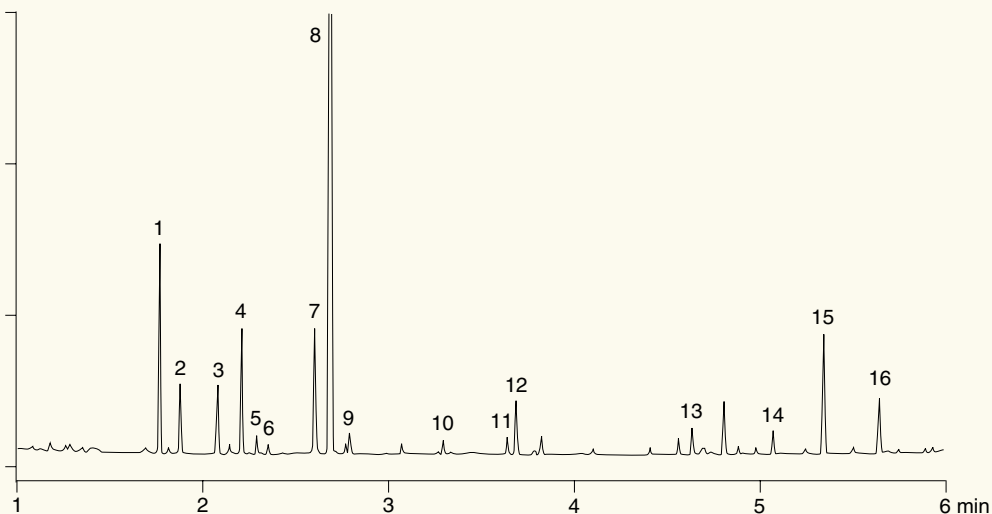
## Free Amino Acids in Beer by GC/FID

### Sample:

Derivatized amino acids in beer.

Norvaline is the internal standard added at 200 µmol/L

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



**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 8psi (60 kPa)

**Pressure Rise:** Constant pressure

**Oven Program:** 32°C/min from 110°  
to 320°C, hold at  
320°C for 1 minute

**Detector:** FID @ 320°C



**Sample:**

Derivatized amino acids from a corn meal hydrolysate sample.  
Norvaline is the internal standard added at 200 µmol/L

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

**Amino Acids  
in Corn Meal  
Hydrolysate  
by GC/FID**

**Kit:** EZ:faast GC/FID Protein Hydrolysate Kit

**Order No.:** KGO-7167

**Injection:** Split 1:15 @ 250°C, 2.5µL

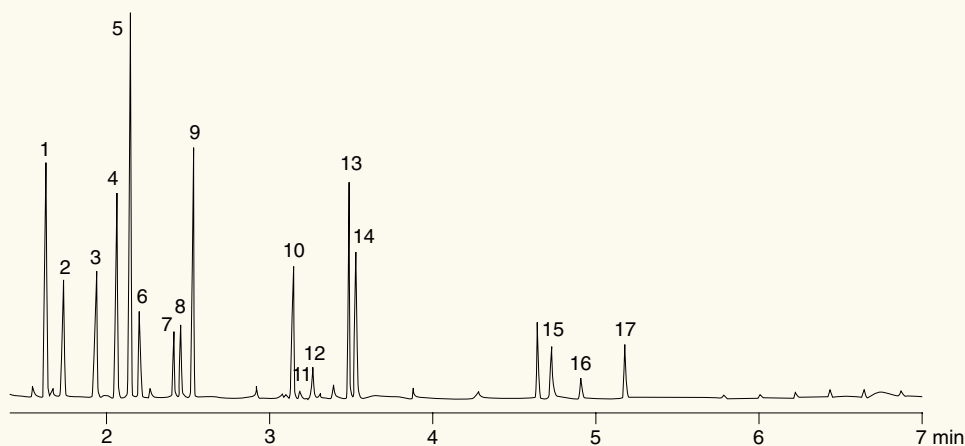
**Carrier Gas:** Helium 1.5mL/minute (60 kPa) @ 110°C

**Pressure Rise:** 6kPa/min

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

**Detector:** FID @ 320°C

App ID 14346



**kit components**



Internal Standard Washing Solution (Reference Sample Clean-up)  
Eluting Medium I (Sample Clean-up)  
Eluting Medium II (Sample Clean-up)  
Organic Solution I (Derivatization)  
Organic Solution II (Derivatization)  
Acid Solution (Derivatization)



Amino Acids Standard Solution



400 Sample Preparation Vials

Microdispenser for Organic Reagents 4 and 5



0.6mL Syringe for SPE amino acid elution



Vial Rack for Sample Preparation



1.5mL Syringe for SPE loading & washing



Zebron™ ZB-AAA GC Columns or AAA HPLC Column



384 SPE Sorbent Tips  
SGE GC FocusLiners (Not Shown)

EZ:faast workstation



## Frequently Asked Questions

### Q: For how long are the derivatized amino acids stable?

A: Amino acids derivatized by the EZ:faast method are stable for up to a day at room temperature and for several days if refrigerated.

### Q: How many samples can be analyzed with the EZ:faast kits?

A: 384 samples can be analyzed with the reagents and supplies of one kit.

### Q: How long will the EZ:faast GC and LC column last?

A: We have found that the GC and LC columns will last for the duration of the kit. For best results columns should be replaced with each new kit.

### Q: What is the shelf life of the reagents?

A: EZ:faast reagents have a guaranteed shelf life of 12 months if properly stored as indicated on the bottle in a refrigerator or freezer.

### Q: What are the derivatization reagents and reaction?

A: The derivatization reagents and reaction are proprietary. The reaction derivatizes both the amine and carboxyl groups of the amino acids forming a highly stable derivative.

## Amino Acid Analysis Kits

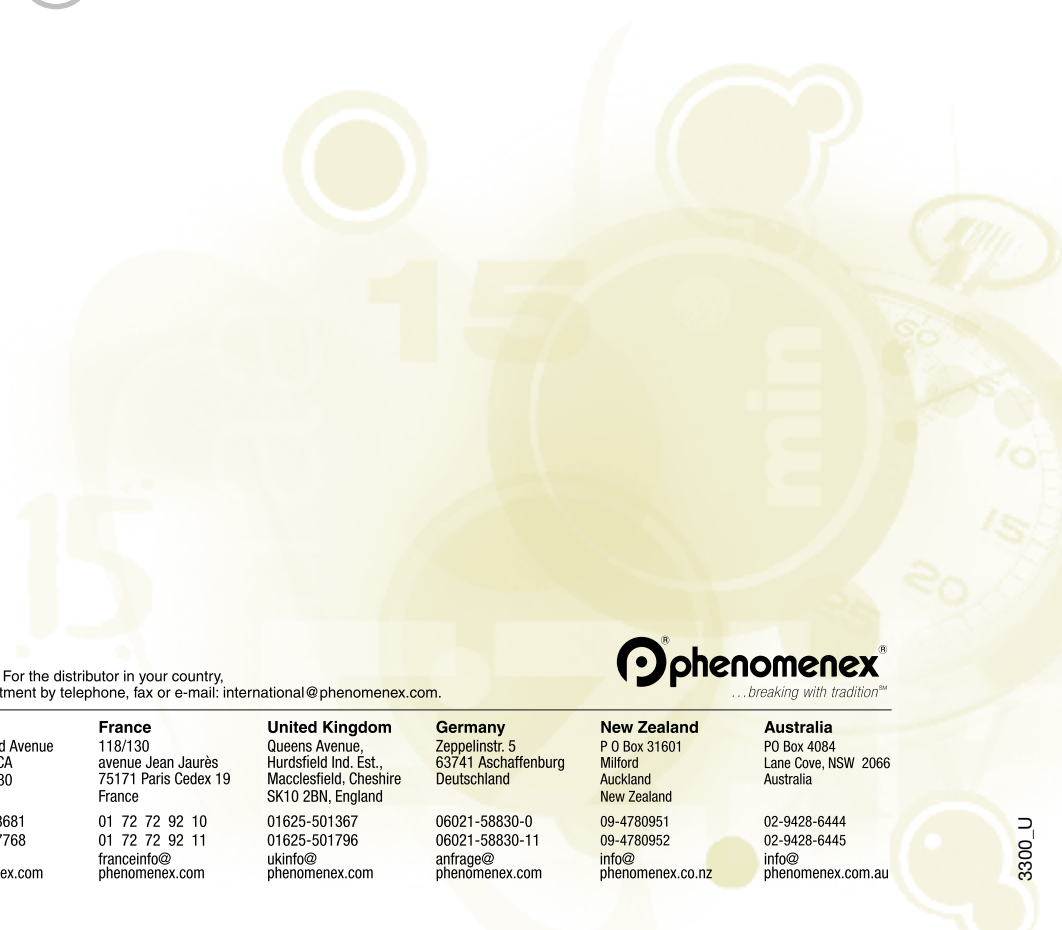
Each kit includes: ZB-AAA GC column, or AAA LC column, sample prep and derivatization reagents, sample prep vials, AA standards, SPE pipette tips, vial rack, and microdispenser.

| Order 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 2.0mm column | ea   |       |
| KH0-7338  | LC/MS Free (Physiological) Amino Acids Kit with 250 x 3.0mm column | ea   |       |
| KH0-7339  | LC/MS Protein Hydrolysates Kit with 250 x 2.0mm column             | ea   |       |
| KH0-7340  | LC/MS Protein Hydrolysates Kit with 250 x 3.0mm column             | ea   |       |
| AG0-7184  | Free (Physiological) Amino Acid Standards (SD1, 2, 3) 2mL/vial x 2 | ea   |       |
| AG0-7263  | Protein Hydrolysate Standard (SD) 2mL/vial x 2                     | ea   |       |

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| <b>fax:</b> 310-328-7768   | 310-328-7768   | 310-328-7768   | 01 72 72 92 11   | 01625-501796  | 06021-58830-11   | 09-4780952   | 02-9428-6445   |
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