

Amino Acids: Biofluids, cell cultures, or Tissues

Service Code: AAA

Reference: <http://www.fortunesci.com/image/download2/USER%20GUIDE/EZfaast%20Guide.pdf>

Summary: Amino acids are analyzed using the Phenomenex EZfaast kit. Samples are extracted, semi-purified, derivitized and measured by EI-GCMS using norvaline as an internal standard for normalization. See reference for User Guide and analytical details. Analytes are reported as uM with CV's generally 10%, but Histidine is exceptional with a CV ~30%.

Container: Eppendorf Tube or equivalent

Normal Volume: Plasma (100 ul) Tissue (50-100 mgs); Cells (2E7).

Minimal Volume: Plasma (50 uL) Tissue (30 mg); Cells (~5E6)

Special Handling: If human or primate, note any known presence of infectious agents.

Sample Collection: Snap freeze by liquid nitrogen. For tissues, resect and snap-freeze as soon as practical in tared centrifuge tube. Provide both sample weight and tared vial weight on sample submission

Table I: Analytes Reported. D- and L- enantiomers are not distinguished Arginine, Cysteine, Cystine, and others on request.

Analyte	Abbr.	Pubchem	LOD (uM)
Alanine	A, Ala	5950	
Asparagine	N, Asn	236	
Aspartate	D, Asp	424	
Glutamine	Q, Gln	738	
Glutamate	E, Glu	611	
Glycine	G, Gly	750	
Histidine	H, His	773	
Isoleucine	I, Ile	791	
Leucine	L, Leu	857	
Lysine	K, Lys	866	
Methionine	M, Met	876	
Phenylalanine	F, Phe	994	
Proline	P, Pro	614	
Serine	S, Ser	617	
Threonine	T, Thr	205	
Tryptophan	W, Trp	1148	
Tyrosine	Y, Tyr	1153	
Valine	V, Val	1182	
Sarcosine(N-methyl-glycine)	Sar	1088	
<i>alpha</i> -Aminoisobutyric acid	Aib	6119	
<i>gamma</i> -Hydroxyproline	Hyp	825	
Ornithine	Orn	389	

Materials

1. Agilent 6890 GCMS with 5973 MSD unit, or equivalent
2. EZ:faast analysis kit (Phenomenex)
3. Vortexer
4. Refrigerated centrifuge, capable of 13,000g with eppendorf tube compatible rotor
5. Prepared stock solutions of amino acid standards
6. ice bucket, ice
7. UHP N₂ gas dryer

PROCEDURE

Sample Preparation

1. Prior to analysis, an internal standard (norvaline) is added to 50 µL of serum/plasma to be analyzed.
2. Amino acids in the sample are concentrated by cation exchange solid phase extraction, washed, and are then eluted. The eluent is derivatized by addition of derivatization reagent, followed by vortexing and a 2-minute reaction period.
3. The amino acid derivatives are extracted into an organic layer. This layer is collected and the solvent evaporated under N₂ gas stream, and the residue re-constituted in solvent for GC-MS analysis.

GCMS Procedure

1. Agilent 6890 GC with 5973 MSD: EI-70eV, source-240 °C, Auxiliary-310°C
2. GC column: amino acid column, 10m x0.25mmx0.25um (part of EZ:faast Kit)
3. Method file for data acquisition and analysis (part of EZ:faast Kit)
4. Carrier gas: He
5. Gradient: flow rate: 1.1ml/min constant
6. Autosampler: room temperature
7. Injector: 250°C, 1 uL injection with 1:10 split ratio