



Amino Acid Analysis

Analysis of amino acids is required in several areas of research, and it is a fundamental tool in product analysis. The application imposes different requirements on the analytical method because the amino acids play different roles.

- Amino acids are the basic constituents of proteins. Qualitative and quantitative analysis of the amino acid composition of hydrolyzed samples of pure proteins or peptides is used to identify the material and to directly measure its concentration.
- Amino acids are also intermediates in metabolic pathways, often not directly involving proteins. The amino acids are measured as elements of physiological and nutritional studies. This has proven particularly important in monitoring the growth of cells in cultures, as used in the production of biopharmaceuticals.
- Similar considerations lead to the analysis of foods and feeds to ensure that nutritional requirements are met.

AccQ•Tag™ Ultra Chemistries used in the UPLC Amino Acid Analysis Solution are a comprehensive and fully tested set of reagents, columns, and eluents optimized for use with the ACQUITY UPLC® System. With the system's superior resolution of all amino acids, you will confidently achieve peak identification and quantitation, which in turn assures the accuracy of your results. This chemistry is based on Waters' widely-used and understood AccQ•Tag™ derivatization method. Primary and secondary amino acids react with a single reagent, AccQ•Fluor™, in a high-throughput batch process resulting in exceptionally stable derivatives. High resolution separations are achieved using pre-qualified AccQ•Tag™ Ultra UPLC columns and mobile phases. Derivatized amino acids are quantified to sub-picomole levels with single wavelength UV detection.

The UPLC Amino Acid Analysis has the following features:

- Method robustness minimizes variability.
- Better resolution translates into accurate results.
- UPLC delivers greater throughput than traditional HPLC.
- Protein characterization accelerates for comparison of amino acid composition deduced from the gene sequence for a product or its natural counterpart.

- Monitoring cell culture media for critical nutrients allows you to optimize expression efficiencies and to make timely product harvesting decisions.

We are using a 9.5 min method for separation and perform either free amino acid analysis (client extracted) or 6M HCl hydrolysis. We recommend samples be run in triplicate for appropriate statistical analysis.

Please follow the guidelines below for submitting your samples.

FREE AAA:

For free amino acids, samples must be extracted by the customer.

We recommend the procedure below:

Hacham et al., 2002 Plant Physiology 128: 454-462):

- Freeze 150 mg of tissue in liquid nitrogen.
- Grind with mortar and pestle in the presence of 600 µL of water:chloroform:methanol (3:5:12 v/v).
- Transfer to 1.5 mL tube and centrifuge at full speed for 2 minutes.
- Collect the supernatant into a 2 mL tube.
- Re-extract the residue with another 600 µL of water:chloroform:methanol extraction solvent.
- Centrifuge at full speed for 2 minutes.
- Collect the supernatant and combine with the previous one in the 2 mL tube.
- Add 300 µL of chloroform and 450 µL of water to the combined supernatants.
- Centrifuge at full speed for 2 minutes.
- Collect the upper water:methanol phase and transfer to a fresh tube.
- Speed vac to dry.
- Store at -20 °C, and ship on dry ice to the facility.

For free amino acids, we have a method calibrated from 5pmol-50pmol of each of the following amino acids: **His, Asn, Tau, Ser, Gln, Arg, Gly, Asp, Glu, Thr, Ala, GABA, Pro, Cys, Lys, Tyr, Met, Val, NorVal, Ile, Leu, Phe, Trp.**

HYDROLYZED AAA:

For solid samples to be analyzed after hydrolysis, weigh ~3mg of fresh or dry ground tissue and place in the bottom of a wide bottom 350 µL clear glass insert (ex: XPERTEK p/n 951710) in a wide mouth 2 mL autosampler vial with screw cap (ex: XPERTEK p/n 954423). For single peptide or protein sample, we require a minimum of 3 nmoles, as well as a

negative control of the buffer used for the samples. For single protein analysis sample can be sent in 1.5 mL Eppendorf tubes.

Please clearly label your vial/tubes, and include printed information about your samples, including weights.

We will keep the samples stored at -80 °C prior to analysis.

For HAA, we have a method calibrated from 5pmol-50pmol of each of the following amino acids: **Cya****, **His**, **Ser**, **Arg**, **Gly**, **Asp**, **MetSO2****, **Glu**, **Thr**, **Ala**, **Pro**, **Lys**, **Tyr**, **Val**, **Ile**, **Leu**, **Phe**

** Cysteine (Cys) Methionine (Met) and Tryptophan (Trp) are destroyed during hydrolysis with 6MHCl. We include an initial oxidation step using performic acid prior to the standard acid hydrolysis, which yields the acid stable forms cysteic acid (Cya) and methionine sulfoxide (MetSO₂) that can be measured. Tryptophan will not be detected following hydrolysis.