

## Amino Acid Standard Kits

### CONTENTS

- I. INTRODUCTION
- II. RECONSTITUTION RECOMMENDATIONS
- III. STORAGE
- IV. AMINO ACID ANALYSIS - QUICK START PROTOCOL
- V. EXPERIMENTAL CONDITIONS AND REPRESENTATIVE DATA FOR THE UPLC AMINO ACID ANALYSIS
- VI. ORDERING INFORMATION

### I. INTRODUCTION

Amino acid analysis is required in many applications in pharmaceutical and food and feed industries. A variety of standards containing free amino acids are offered for qualitative and quantitative determination of amino acids, method development and troubleshooting of the AccQ•Tag™ Ultra or AccQ•Tag methods. This care and use manual provides informative instructions on how to use these standards.

For more information, please visit [www.waters.com/AAA](http://www.waters.com/AAA).

***This product is for Research Use Only — not for use in diagnostic procedures.***



Figure 1. Amino Acid Standards.

**Table 1. List of Amino Acids in Each Amino Acid Standard**


Amino Acid	Amino Acid Standard p/n: WAT088122	Cell Culture Standard Kit p/n: 186009300	Food & Feed Standard Kit p/n: 186009299	Internal Standard p/n: 186009301
Alanine	●	●	●	
Arginine	●	●	●	
Aspartic acid	●	●	●	
Cystine	●	●	●	
Glutamic acid	●	●	●	
Glycine	●	●	●	
Histidine	●	●	●	
Isoleucine	●	●	●	
Leucine	●	●	●	
Lysine	●	●	●	
Methionine	●	●	●	
Phenylalanine	●	●	●	
Proline	●	●	●	
Serine	●	●	●	
Threonine	●	●	●	
Tyrosine	●	●	●	
Valine	●	●	●	
Taurine		●	●	
HydroxyProline		●		
Asparagine		●		
Glutamine		●		
GABA ( $\gamma$ -Aminobutyric acid)		●		
Tryptophan		●		
Ornithine		●		
AABA ( $\alpha$ -Aminobutyric acid)		●	●	
HydroxyLysine		●		
Methionine Sulfone			●	
Cysteic Acid			●	
Norvaline				●


## II. RECONSTITUTION RECOMMENDATIONS

For general use with UV or fluorescence detection instruments, the following is recommended:

Description	Recommended Supplier
0.1 M HCl	LC-MS-grade HCl is recommended (Honeywell Fluka 35335 or equivalent)



### Cell Culture Standard Kit (p/n: 186009300)

1. Add 1.2 mL of 0.1 M HCl to 17 Amino Acid Hydrolysates Standard (blue vial ) to give a concentration of 1000  $\mu$ M for each amino acid except cystine which has a concentration of 500  $\mu$ M. Vortex for 10 seconds. This standard is stable up to 60 days after reconstitution when stored in a freezer.

2. Add 250  $\mu\text{L}$  of 0.1 M HCl to the additional Cell Culture Standard (white vial ) to give a concentration of 1000  $\mu\text{M}$ . Vortex for 10 seconds. This standard is stable up to 60 days (with the exception of glutamine in the cell culture standard which is stable up to 15 days) after reconstitution when stored in a freezer.
3. Add 250  $\mu\text{L}$  of the prepared hydrolysate standard into the additional cell culture vial to give a 500  $\mu\text{L}$  of 500  $\mu\text{M}$  amino acid except cystine which has a concentration of 250  $\mu\text{M}$ . Use this as the starting stock standard to create a calibration curve. This standard is stable up to 60 days (with the exception of glutamine in the cell culture standard which is stable up to 15 days) after reconstitution when stored in a freezer.

*Note: If the standard solid is found on the septum, invert the vial during vortexing to ensure all the powder is dissolved.*

### Food & Feed Standard Kit (p/n: 186009299)

1. Add 1.2 mL of 0.1 M HCl to 17 Amino Acid Hydrolysates Standard (blue vial ) to give a concentration of 1000  $\mu\text{M}$  for each amino acid except cystine which has a concentration of 500  $\mu\text{M}$ . Vortex for 10 seconds. This standard is stable up to 60 days after reconstitution when stored in a freezer.
2. Add 250  $\mu\text{L}$  of 0.1 M HCl to the additional food and feed standard (green vial ) to give a concentration of 1000  $\mu\text{M}$ . Vortex for 10 seconds. This standard is stable up to 60 days after reconstitution when stored in a freezer.
3. Add 250  $\mu\text{L}$  of the prepared hydrolysate standard into the additional food and feed vial to give a 500  $\mu\text{L}$  of 500  $\mu\text{M}$  amino acid except cystine which has a concentration of 250  $\mu\text{M}$ . Use this as the starting stock standard to create a calibration curve. This standard is stable up to 60 days after reconstitution when stored in a freezer.

*Note: If the standard solid is found on the septum, invert the vial during vortexing to ensure all the powder is dissolved.*

### Example Calibration Standard Preparation

The following Dilution scheme was used to create a series of calibrators. This will be the starting stock used to create a calibration curve.

#### 1. Calibrator 7: 500 $\mu\text{M}$ (250 $\mu\text{M}$ for cystine)

Start with 500  $\mu\text{L}$  of 500  $\mu\text{M}$  vial containing 26 amino acids (cell culture) or 21 amino acids (Food and Feed) once both large vial and small vial from kit are reconstituted and combined.

#### 2. Calibrator 6: 250 $\mu\text{M}$ (125 $\mu\text{M}$ for cystine)

Take 215  $\mu\text{L}$  of calibrator 7 and add to calibrator 6 vial. Add 215  $\mu\text{L}$  of 0.1 M HCl. Mix.

#### 3. Calibrator 5: 100 $\mu\text{M}$ (50 $\mu\text{M}$ for cystine)

Take 150  $\mu\text{L}$  of calibrator 6 and add to calibrator 5 vial. Add 225  $\mu\text{L}$  of 0.1 M HCl. Mix.

#### 4. Calibrator 4: 20 $\mu\text{M}$ (10 $\mu\text{M}$ for cystine)

Take 93  $\mu\text{L}$  of calibrator 5 and add to calibrator 4 vial. Add 372  $\mu\text{L}$  of 0.1 M HCl. Mix.

#### 5. Calibrator 3: 10 $\mu\text{M}$ (5 $\mu\text{M}$ for cystine)

Take 182  $\mu\text{L}$  of calibrator 4 and add to calibrator 3 vial. Add 182  $\mu\text{L}$  of 0.1 M HCl. Mix.


#### 6. Calibrator 2: 2.5 $\mu\text{M}$ (1.25 $\mu\text{M}$ for cystine)

Take 84  $\mu\text{L}$  of calibrator 3 and add to calibrator 2 vial. Add 252  $\mu\text{L}$  of 0.1 M HCl. Mix.

#### 7. Calibrator 1: 0.5 $\mu\text{M}$ (0.25 $\mu\text{M}$ for cystine)

Take 56  $\mu\text{L}$  of calibrator 2 and add to calibrator 1 vial. Add 224  $\mu\text{L}$  of 0.1 M HCl. Mix.

### Norvaline Internal Standard (p/n: 186009301)

Add 6 mL of diluent to the internal standard (red vial ) and vortex for 10 seconds to give a concentration of 250 µM.

For additional information on the internal standard, please reference these documents:

- Hydrolysis and Analysis of Amino Acids from Purified Peptides/Proteins, Foods, and Feeds (715006455)
- Care and Use Manuals for Tecan, Hamilton (720006662EN , 720006661EN)
- UPLC™ Amino Acid Analysis Solution System Guide (71500129702)
- ACQUITY UPLC H-Class and H-Class Bio Amino Acid Analysis System Guide (715006168)

### III. STORAGE



Perform kit inventory on arrival. Do not use this product if its packaging is opened, damaged, items are missing, or beyond expiry date, if applicable. Replacement kit or parts can be ordered by contacting your local sales representative. Please include product part number and lot number for each item requested.

Part Number	Description	Storage After Reconstitution	Initial Storage
186009299	Amino Acid Food Feed Standard Kit	Amino Acid Food and Feed Standard -10 °C to -25 °C for up to 60 days.	Freeze on arrival, -10 °C to -25 °C.
		Amino Acid 17 Hydrolysate Standard -10 °C to -25 °C for up to 60 days.	
186009300	Amino Acid Cell Culture Standard Kit*	Amino Acid Cell Culture Standard -10 °C to -25 °C for up to 60 days.*	Freeze on arrival, -10 °C to -25 °C.
		Amino Acid 17 Hydrolysate Standard -10 °C to -25 °C for up to 60 days.	
WAT088122	Amino Acid Standard	N/A	Freeze on arrival, -10 °C to -25 °C.
186009301	Amino Acid Internal Standard – Norvaline	-10 °C to -25 °C for up to 60 days.	Freeze on arrival, -10 °C to -25 °C.



\* Glutamine in the cell culture kit degrades overtime after reconstitution. Concentrations for glutamine listed on the product are valid up to 15 days after reconstitution when stored in a freezer.



Some products may be classified as hazardous and are intended for use by professional laboratory personnel trained in the competent handling of such materials. Responsibility for the safe use of products rests entirely with the purchaser and user. The Safety Data Sheet (SDS) containing GHS information for this product is available at [www.waters.com/sds](http://www.waters.com/sds).

## IV. AMINO ACID ANALYSIS – QUICK START PROTOCOL

### Preparation of AccQ-Tag Ultra Reagent



1. Preheat a heating block to 55 °C.
2. Tap vial 2A (AccQ-Tag Ultra Reagent powder) to ensure all reagent is at bottom of vial.
3. Draw 1 mL of AccQ-Tag Ultra Reagent Diluent from vial 2B.
4. Transfer to vial 2A.
5. Vortex for 10 seconds.
6. Heat on top of heating block, vortex occasionally, until the powder dissolves.  
Do not heat longer than 15 minutes.

### Derivatization of Amino Acids

1. Add 70 µL of borate buffer to the sample and mix.
2. Add 10 µL of standard/sample to the sample.
3. Add 20 µL of AccQ-Tag reagent to the sample.
4. Vortex mix the sample for 20 seconds.
5. Heat the samples for 10 minutes at 55 °C then allow to cool for three minutes.
6. Vortex mix the sample for 20 seconds, and transfer sample for analysis.

Unused components and wastes may be classified and regulated as Hazardous Wastes.  
To determine proper disposal, consult local regulations.

## V. EXPERIMENTAL CONDITIONS AND REPRESENTATIVE DATA FOR THE UPLC AMINO ACID ANALYSIS

*Note: Purchase of the UPLC Amino Acid Analysis Solutions is required for chromatographic instructions and support.*

### AccQ-Tag profiling method – cell culture

LC system:	ACQUITY™ UPLC H-Class Bio with TUV
Sample temp.:	20 °C
Column:	AccQ-Tag Ultra, 1.7 µm, 2.1 x 100 mm
Column temp.:	43 °C
Flow rate:	700 µL/min
Injection volume:	1 µL
UV detection:	260 nm
Mobile phase A:	100% AccQ-Tag Ultra eluent A
Mobile phase B:	90:10 water:AccQ-Tag Ultra eluent B
Mobile phase C:	100% HPLC-grade water
Mobile phase D:	100% AccQ-Tag Ultra eluent B

**AccQ-Tag profiling method - food and feeds**

LC system:	ACQUITY UPLC H-Class Bio with TUV
Sample temp.:	20 °C
Column:	AccQ-Tag Ultra, 1.7 µm, 2.1 x 100 mm
Column temp.:	49 °C
Flow rate:	700 µL/min
Injection volume:	1 µL
UV detection:	260 nm
Mobile phase A:	100% AccQ-Tag Ultra eluent A
Mobile phase B:	90:10 water:AccQ-Tag Ultra eluent B
Mobile phase C:	100% HPLC-grade water
Mobile phase D:	100% AccQ-Tag Ultra eluent B

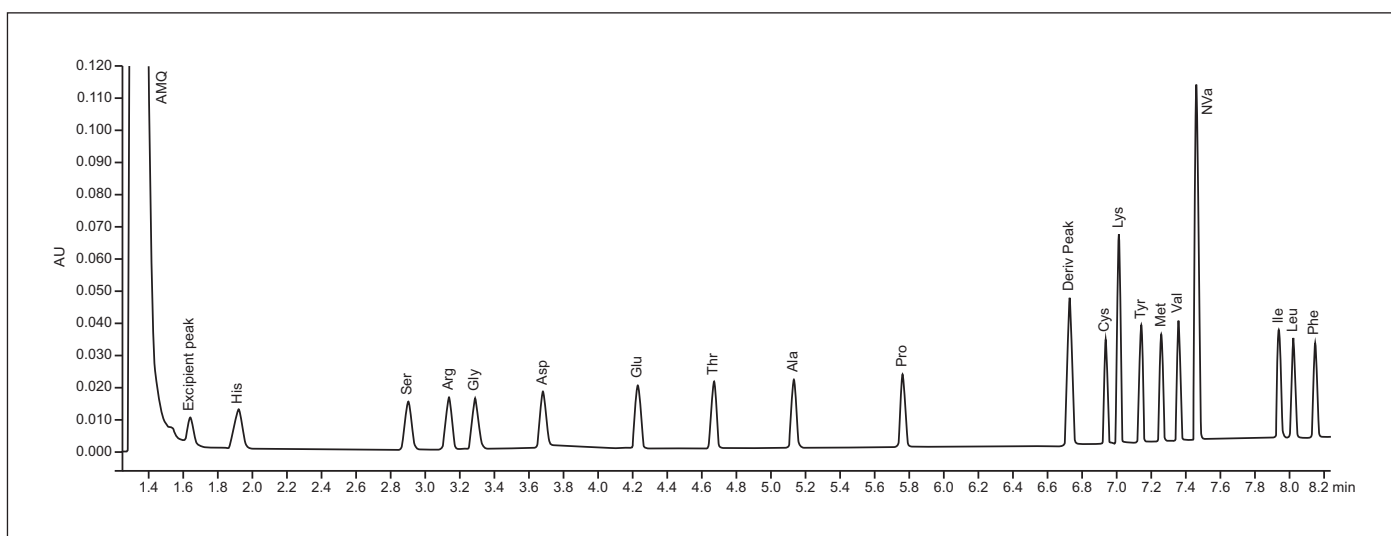


Figure 14. Separation of 10 pmols of the hydrolysate standard spiked with 23.5 pmols of Nva on column.

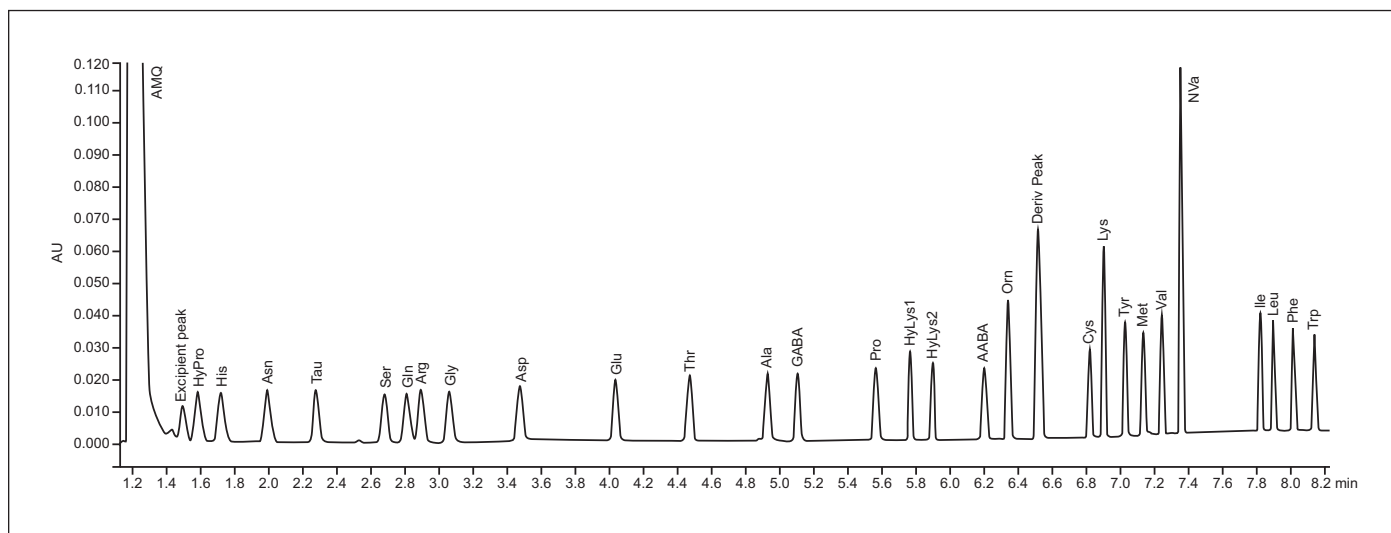


Figure 15. Separation of 10 pmols of the cell culture standard spiked with 23.5 pmols of Nva on column.

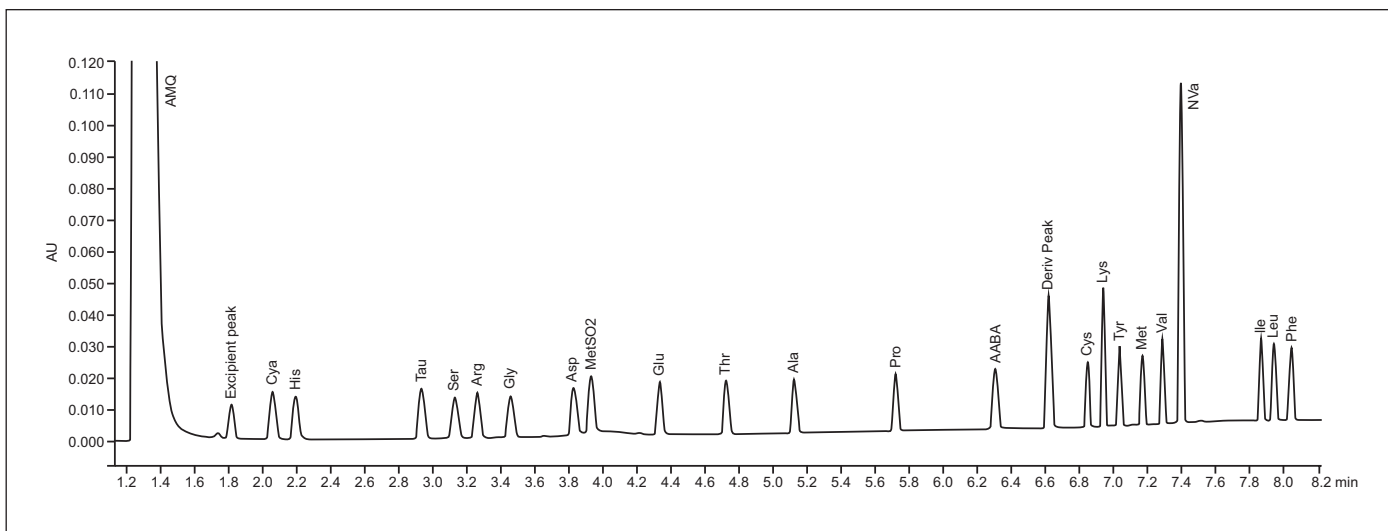


Figure 16. Separation of 10 pmols of the food and feed standard spiked with 23.5 pmols of Nva on column.

## VI. ORDERING INFORMATION

Product Description	Part Number
<b>Amino Acid Standard</b> 10 x 1 mL ampules of unlabeled amino acid standards	WAT088122
<b>Amino Acid Cell Culture Standard Kit</b> 2 vials contain 17 amino acids 8 vials contain 9 cell culture supplemental amino acids	186009300
<b>Amino Acid Food and Feed Standard Kit</b> 2 vials contain 17 amino acids 8 vials contain 4 food and feed supplemental amino acids	186009299
<b>Amino Acid Internal Standard - Norvaline</b> 1 vial	186009301

Qty: 1



**Waters™**

Waters, The Science of What's Possible, AccQ-Tag, ACQUITY, and UPLC are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.

©2024 Waters Corporation. Produced in the U.S.A. January 2024 720006663EN Rev B KK-PDF



**Waters Corporation**  
34 Maple Street  
Milford, MA 01757 U.S.A.  
T: 1 508 478 2000  
F: 1 508 872 1990  
[waters.com](http://waters.com)