

Amino Acids Analysis



Introduction

The first amino acids, known as asparagine was isolated and discovered from asparagus juice in 1806. Since then, amino acids has become an indispensable component in our daily life with four major functions, that is to use it as a nutrient, flavor component, physiological effects and for reactivity.

Amino acids that cannot be synthesized in our bodies are essential amino acids as food nutrients. In Japan, it is expected that people will practice self-medication since several system was implemented such as Food for Specified Health Uses System (1991), the Food with Health Claims System (2002), and the Food with Functional Claims System (2015).

In addition, Foods with Functional Claims System is a notification system which makes it easy for new companies to enter the market. The food market is expected to expand as the number of Food with Functional Claims has already increased rapidly, with 815 products (March 2017), compared to Food with Health Claims with 1,127products(March 2017).

The functions of amino acids as nutrients are already widely recognized, and there are a wide variety of foods containing amino acids on the market, such as beverages, energy drinks, and jellies. It is expected that many new products containing amino acids will be invented in the future.

It has been reported that changes in amino acid concentrations in the body are associated with various pathological conditions. For example, amino acid analysis in blood is used for newborn screening tests and disease risk detection services.

Since there are 20 types of amino acids that make up proteins, separation analysis is used in most analytical methods. And liquid chromatography (HPLC) is the most common method.

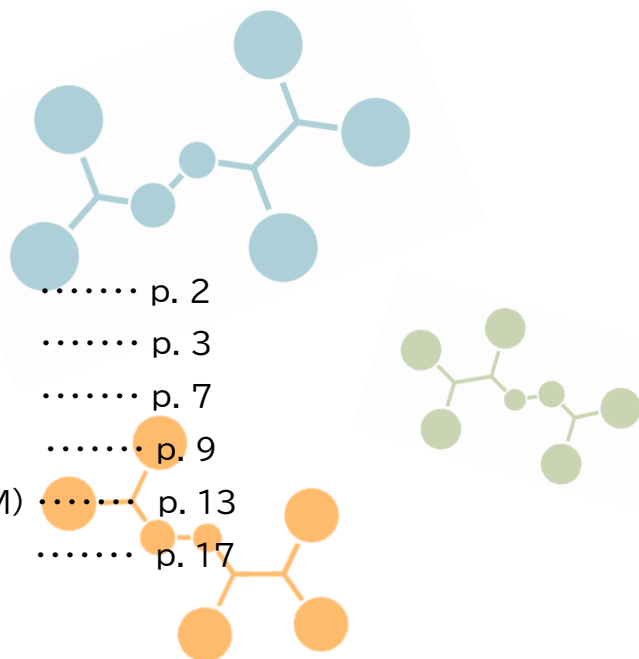
FUJIFILM Wako has a wide range of reagents for amino acid analysis (derivatization reagents, standard solutions, and buffer compatible with various automatic analyzers).

References

- Miyano, H.: *Wako Junyaku Jiho*, 79 (1), 2(2011).
Suto, Y.: *Wako Junyaku Jiho*, 85 (4), 6 (2017).
Tsunoda, M., Sumida, Y.: *Wako Junyaku Jiho*, 86 (4), 9 (2018).

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Wako-blog

Wako-blog introduces a wide range of useful information such as the frontline research, technical reports, and tips for experiments. In Wako-blog, various articles are posted regardless of the field such as life science, organic synthesis, analytical chemistry.

<https://labchem-wako.fujifilm.com/jp/siyaku-blog/027264.html>

About Amino Acid Analysis

Derivatization in amino acid analysis

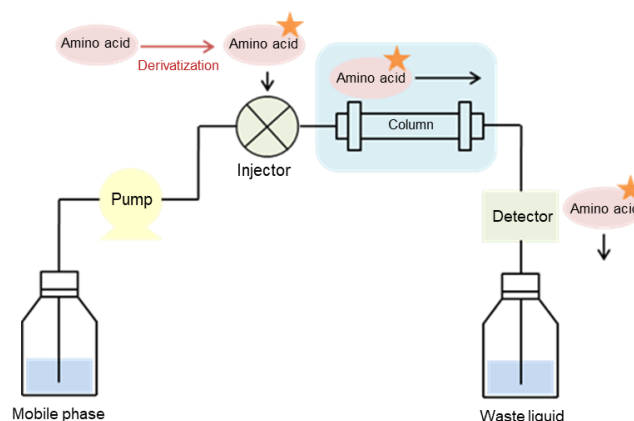
Amino acids are compounds that absorb less ultraviolet. Derivatization is significant in order to detect amino acids by HPLC with high sensitivity.

Typical derivatization methods include [i] pre-column derivatization method by which analytes are transformed into substances suitable for separation and detection before column separation, and [ii] post-column derivatization method by which analytes are transformed into substances suitable for detection after column separation.

[i] Pre-column derivatization method

APDS method (p.3) / PTC method (p.7)

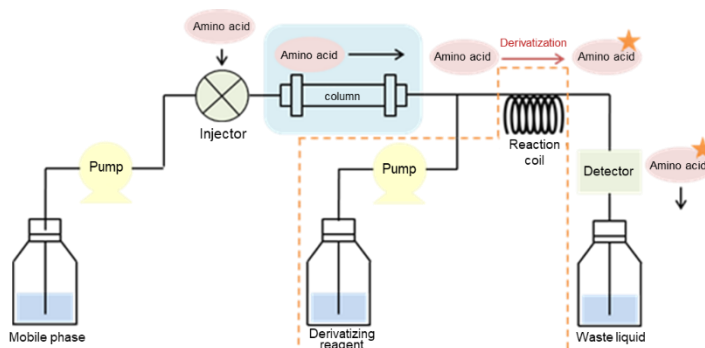
The pre-column derivatization method transforms analytes into substances that are suitable for separation and detection before column separation. As an advantage of this method, consumption of reagents is less. Besides, reagents can be selected according to the type of detector and it can analyze with high sensitivity.



[ii] Post-column derivatization method

Ninhydrin method (p.9)

The post-column derivatization method is a method whereby the analyte is derivatized and led to the detector after column separation. It has the advantages of automated reactions, excellent quantitation and reproducibility, and does not be affected by the sample matrix since the sample components are separated by the column before the reaction.



	Pre-column derivatization method	Post-column derivatization method
Equipment	It can analyze with a general HPLC system but requires special equipment for automation.	Automation is simple with dedicated equipment.
Reagents	Consumption of reagent is small. A wide range of derivatizing reagents are available depending on detector type.	Consumption of reagent is large. Available derivatizing reagents are limited.
Sensitivity and quantification	Highly sensitive analysis is feasible but if derivatized products are unstable it may affect quantitative results.	Highly sensitive analysis may not be feasible, but this method is excellent in quantification and reproducibility.
Reverse-phase HPLC	Applicable	Non-applicable
Impact of sample matrix	Efficiency of derivatization reaction may be affected by sample matrix.	The reaction with derivatizing reagents is not affected by the sample matrix because components in the sample are separated by the column before reaction.

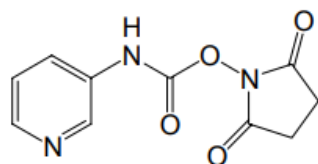
APDS method

Pre-column derivatization method

The APDS method is a method where amino acids are derivatized by APDS (3-Aminopyridyl-*N*-hydroxysuccinimidyl Carbamate) which enable to detect with MS.

Derivatization with APDS followed by separation and detection by LC/MS allow distinction of respective amino acids by m/z even if the retention times are the same and, thus, the analysis time can be shortened significantly.

In addition, APDS is designed to increase the ionization efficiency, allowing highly sensitive amino acid analysis in LC/MS/MS, especially by triple quadrupole.



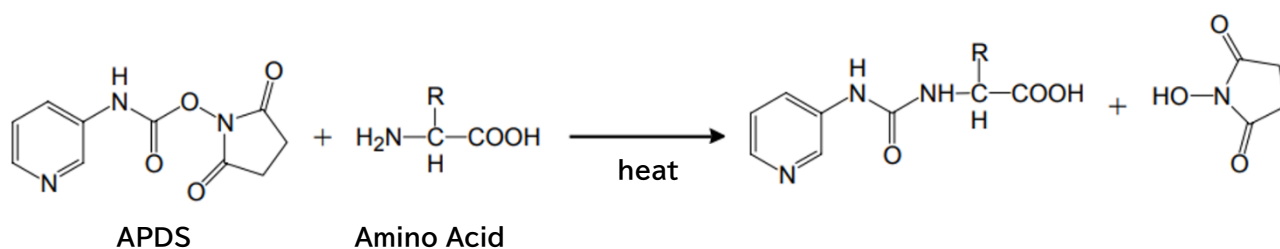
APDS

Features

- APDS have an active carbamate, and any of the compounds reacts under a certain condition (under weak alkaline condition, 55°C, 10 minutes).
- The introduction of an aminopyridyl group increases the hydrophobicity of the derivatives, making it easier to retain and separate by reverse-phase HPLC.
- Due to the high ionization efficiency of the aminopyridyl group, it is detected with the mass spectrometer with high sensitivity.
- A regular product ion is generated which allows highly selective detection of common fragment ions ($m/z = 121$) derived from APDS.

Protocol

When a reaction reagent is added to the sample under alkaline conditions and heated, APDS (3-Aminopyridyl-*N*-hydroxysuccinimidyl carbamate) binds to the amino groups to form derivatized product. This solution is separated by liquid chromatography, and the area values of the amino acid derivatives are detected for each mass-to-charge ratio. From the obtained area value and the ratio of the area value of the standard solution, the concentration of each amino acid in the sample is calculated.



Example of use [1] Quantitation of amino acids in beverages

This article was written by Yuki Suto, FUJIFILM Wako Pure Chemical Corporation, for Vol. 85, No. 4 (October 2017) of *Wako Junyaku Jiho*.

Derivatization with 3-Aminopyridyl-*N*-Hydroxysuccinimidyl Carbamate (APDS)

Derivatization methods for amino acid analysis in HPLC have existed for many years and there are numerous derivatization reagents, including ninhydrin and *o*-phthalaldehyde. The amino acid derivatization method with APDS is an advantageous technique for reverse-phase LC/MS(/MS) because it increases the hydrophobicity and ionization efficiency of amino acids.^{1,2)}

This method has been used in "AminoIndex[®] cancer screening (AICS)" of Ajinomoto Co., Inc.³⁾, and also in the food analyses, there are analysis examples using UF-Amino Station, equipment dedicated to amino acid analysis.⁴⁾

Wakopak[®] Ultra APDS TAG[®] is a dedicated UHPLC column that enables the resolution and analysis of APDS-amino acid derivative using general-purpose LC/MS/MS equipment. We will introduce analyses of amino acids in commercial beverages using this column.

Experimental Method and Results

The flow of sample pretreatment and derivatization is shown in Figure 1, and the LC/MS/MS conditions are shown in Table 1. Quantification was performed by the internal standard method. The commercial beverage used in this study was labeled as containing 6 amino acids (glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), and arginine (Arg)). Amino acids from No. 13 to No. 20 in Table 1 are not contained in this commercial beverage.

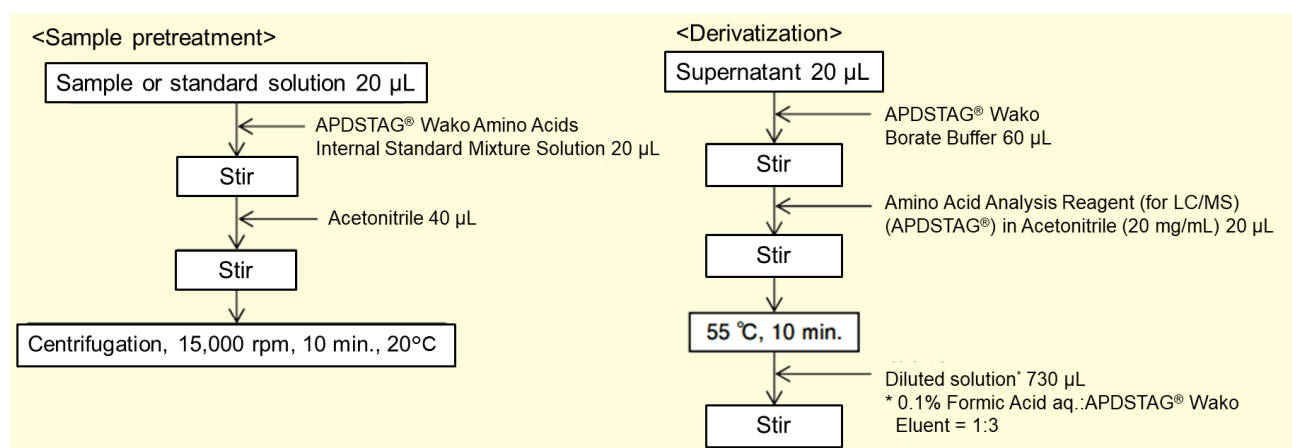


Figure 1. The flow of sample pretreatment and derivatization.

Table 1. LC/MS/MS conditions

Equipment	Prominence LC-20A XR (Shimadzu Co., Ltd.)	No.	Amino acid (Abbreviation)	Molecular weight	Monitor ion (m/z)	
Column	Wakopak [®] Ultra APDS TAG [®] φ 2.1 × 100 mm (W)				Precursor	Product
Guard filter	SunShell RP guard filter (ChromaNik Technologies Inc.)	1	Glycine (Gly)	75.1	196.1	121.0
A conc.	APDS TAG [®] Wako Eluent	2	Glycine - ¹³ C ₂ , ¹⁵ N (Gly-IS)	78.1	199.1	121.1
B conc.	60 % Acetonitrile	3	Alanine (Ala)	89.1	210.1	121.0
Gradient	0min. (A:B=94:6) → 0.05min. (92:8) → 1.70min. (92:8) → 1.71min. (88:12) → 4.95min. (70:30) → 5.95min. (40:60) → 5.96min. (5:95) → 6.70min. (5:95) → 6.71min. (94:6) → 12min. (94:6)	4	Alanine - ¹³ C ₃ (Ala-IS)	92.1	213.1	121.1
Flow rate	0.3 mL/min.	5	Valine (Val)	117.2	238.1	121.0
Column temperature	40 °C	6	Valine - ¹³ C ₆ , ¹⁵ N (Val-IS)	123.1	244.1	121.1
Injection	1 μL	7	Leucine (Leu)	131.2	252.1	121.0
Equipment	LCMS-8030 Plus (Shimadzu Co., Ltd.)	8	Isoleucine (Ile)	131.2	252.1	121.0
Ionization	ESI positive	9	Leucine-5,5,5-d ₃ (Leu-IS)	134.2	255.1	121.1
Measurement mode	MRM	10	Isoleucine - ¹³ C ₆ , ¹⁵ N (Ile-IS)	138.1	259.1	121.1
DL temperature	250 °C	11	Arginine (Arg)	174.2	295.1	175.1
Nebulizer gas flow rate	1.5 L/min.	12	Arginine - ¹⁵ N ₄ (Arg-IS)	178.2	299.1	179.1
Heat block temperature	250 °C	13	Sarcosine (Sar)	89.1	210.0	121.0
Drying gas flow rate	10 L/min.	14	Hydroxyproline (Hypro)	131.1	252.1	121.0
		15	Asparagine (Asn)	132.1	253.1	121.0
		16	Aspartic acid (Asp)	133.1	254.1	121.0
		17	Glutamine (Gln)	146.1	267.1	121.0
		18	Glutamic acid (Glu)	147.1	268.1	121.0
		19	α-Aminoadipic acid (α-AAA)	161.2	282.1	121.0
		20	Citrulline (Cit)	175.2	296.3	121.0

The MRM chromatogram of 6 amino acids in the commercial beverage is shown in Figure 2.

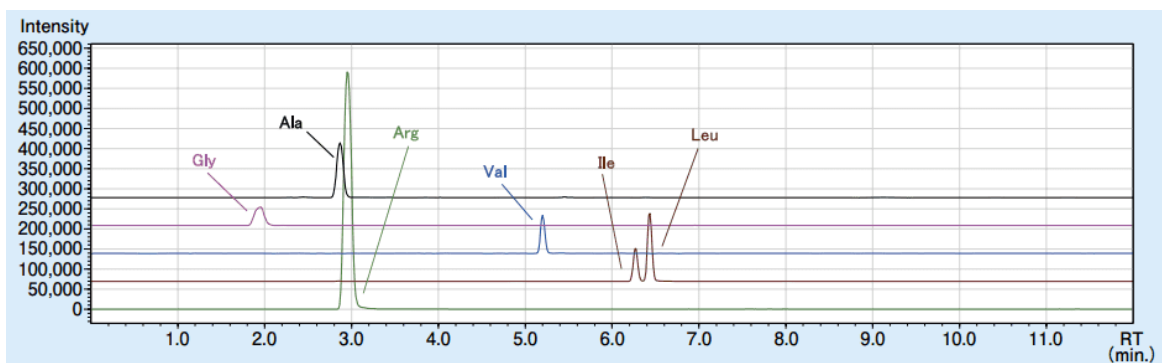


Figure 2. The MRM chromatogram of 6 amino acids in the commercial beverage

Next, the analytical standards of amino acids were spiked with the commercial beverage, and the spike and recovery test was performed. The MRM chromatogram of the commercial beverage after spiking the analytical standards is shown in Figure 3, and the results of the spiking and recovery test are shown in Figure 4. The recovery rate was 99-103%, showing a high recovery rate.

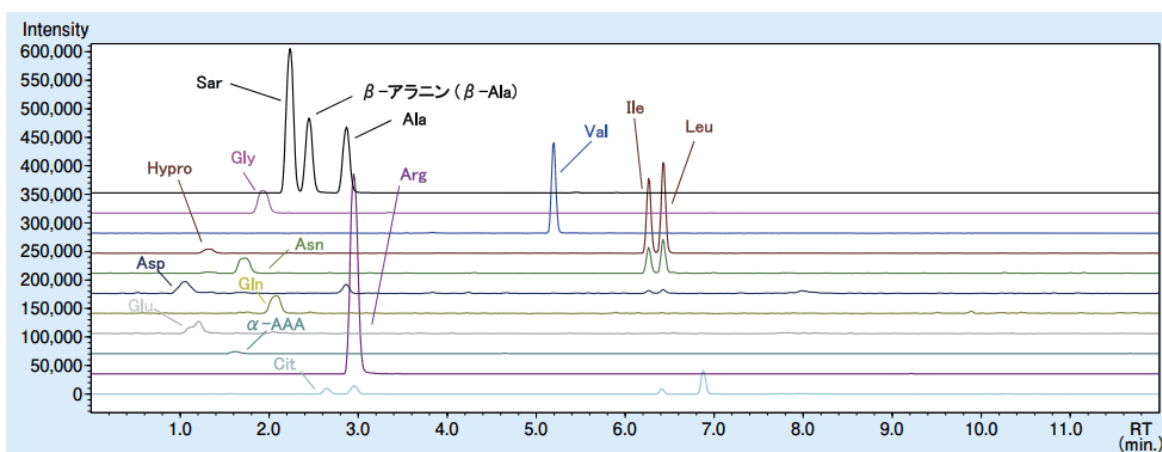


Figure 3. MRM chromatogram of commercial beverage after spiking analytical standards

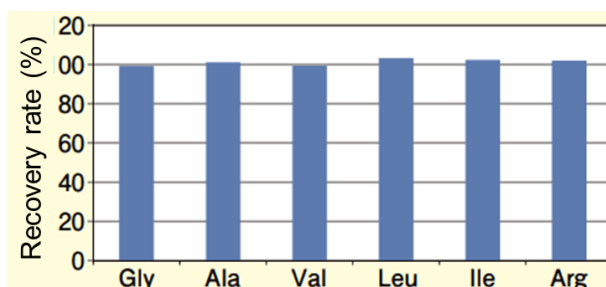


Figure 4. Results of spiking and recovery test

Based on the results of this study, the quantification of amino acids by APDS derivatization using Wakopak® Ultra APDS TAG® is considered effective. Although the results presented here are only for the beverage, it is considered useful also in the analysis of samples including other foods and culture fluids. Please utilize this UHPLC column for the quantification of amino acids.

Acknowledgements

We would like to express our deep appreciation to all members of Ajinomoto Co., Inc. for their cooperation in the study of Wakopak® Ultra APDS TAG®.

References

1. Patent No. 4453363.
2. Miyano, H.: *Wako Junyaku Jiho*, **79** (1), 2 (2011).
3. Imaizumi, A.: *KAGAKU TO SEIBUTSU*, **53** (3), 192 (2015).
4. Watanabe K. *et al.*: *Shimadzu hyoron (Shimadzu review)*, **69** (1, 2), 47 (2012).

Example of use [2] LC/MS high-speed amino acid analysis

APDS can be used as a derivatization reagent dedicated to the LC/MS high-speed amino acid analysis system of Shimadzu Corporation. This system enables simultaneous analysis of 38 or more amino acid-related substances* in just 9 minutes. In addition, since the derivatization reaction is automated, no complicated pretreatment operation is required.

*At least 38 amino acid-related substances including anserine, ctrulline, taurine, GABA (γ -aminobutyric acid), etc. in addition to 20 major amino acids.



For details, please see the website of Shimadzu Corporation
<https://www.shimadzu.com/an/products/liquid-chromatography/hplc-system/uf-amino-station/index.html>

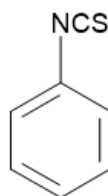
Products used in the APDS method

	Code No.	Product Name	Grade	Volume
Derivatizing reagent	014-23841	Amino Acid Analysis Reagent (for LC/MS)(APDSTAG®)	for Amino Acid Automated Analysis	100 mg
HPLC column	235-64051	Wakopak® Ultra APDS TAG® ϕ 2.1mm×100mm (D)	-	1 EA
Eluent	010-23061	APDSTAG Wako Eluent	for Amino Acid Automated Analysis	1 L
	019-23151	APDSTAG Wako Borate Buffer		1 L
	012-19851	Acetonitrile	for LC/MS	1 L
	018-19853			3 L
Mixture standard solution	016-28161	Amino Acids Mixture Standard Solution, Type AN (High Range)	for Amino Acid Automated Analysis	1 mL×5 A
	010-28164			5 mL
	012-28141	Amino Acids Mixture Standard Solution, Type B (High Range)		1 mL×5 A
	016-28144			5 mL
	019-28151	Amino Acids Mixture Standard Solution, Type H (High Range)		1 mL×5 A
	013-28154			5 mL
	015-27891	Amino Acids Mixture Standard Solution, Type AN [CRM]		1 mL×5 A
	011-27871	Amino Acids Mixture Standard Solution, Type B [CRM]		1 mL×5 A
	018-27881	Amino Acids Mixture Standard Solution, Type H [CRM]		1 mL×5 A
	017-27851	APDSTAG® Wako Amino Acids Mixture Standard Solution No.1 [CRM]		2 mL×5 A
014-27861	APDSTAG® Wako Amino Acids Mixture Standard Solution No.2 [CRM]	2 mL×5 A		
Internal standard mixture solution	293-73701	APDSTAG® Wako Amino Acids Internal Standard Mixture Solution	for Amino Acid Automated Analysis	1 set

PTC method

Pre-column derivatization method

PTC (Phenylthiocarbamide) derivatization is a method derivatizing with the phenylisothiocyanate (PITC), which reacts with primary and secondary amines. The PTC method has the advantage that all the derivatized amino acids have almost the same molecular extinction coefficient. Simultaneous HPLC analysis of multi-amino-acids requires gradient elution. Combining Wakopak® Wakosil®-PTC with a dedicated eluent simplifies gradient conditions and enables highly reproducible separation analysis of amino acids.



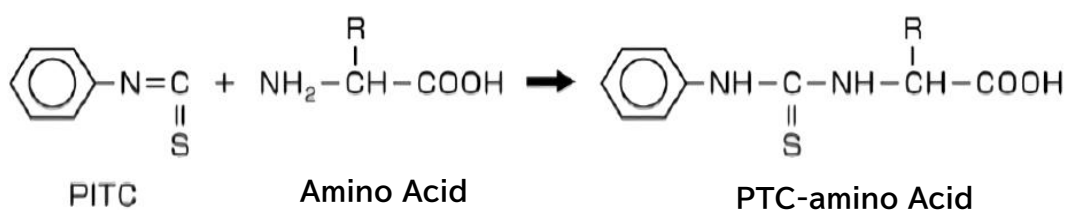
PITC

Phenyl Isothiocyanate

Derivatization method (our recommended method)

1. 10 μL of sample (10 pmol-500 nmol) and amino acid solution (25 nmol) are taken by an Eppendorf tube.
2. Dry under reduced pressure.
3. Add 20 μL mixture of ethanol/purified water/triethylamine (TEA) = (2/2/1) and stir.
4. Dry under reduced pressure.
5. Add 20 μL mixture of ethanol/purified water/TEA/PITC = (7/1/1/1) and stir.
6. Allow to react for 20 minutes at room temperature.
7. Dry under reduced pressure.
8. Store frozen until analysis.
9. Dissolve in PTC-Amino Acids Mobile Phase A for analysis.

*For standard amino acids dissolved in 1 mL, a 10 μL injection corresponds to 250 pmol. For the sample, dissolved in an appropriate amount of PTC-Amino Acids Mobile Phase A.



Notes

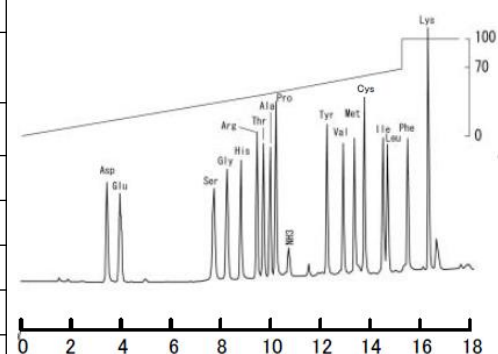
- When derivatizing a sample, the standard solution needs to be derivatized at the same time, and the analysis results of the standard solution are used to perform quantitative calculations of amino acids in the sample.
- Derivatized samples can be used for about 3 months under dry and frozen (-20°C) conditions. The solution dissolved in PTC-Amino Acids Mobile Phase A can be stored frozen and used for several days.

Example of use: Amino Acids Mixture Standard Solution, Type H

▼ Analytical conditions

Column	Wakopak® Wakosil-PTC (4.0 mm x 200 mm)
Eluent	A) PTC-Amino Acids Mobile Phase A B) PTC-Amino Acids Mobile Phase B
Gradient condition	0→15 min. B conc. 0→70%
Flow rate	1.0 mL/min
Column temperature	40°C
Injection volume	1 µL
Detection	UV 254 nm
Sample	PTC-Amino Acids solution. (250 pmol)

▼ Chromatogram



Products used in the PTC method

	Code No.	Product Name	Grade	Volume	
Derivatizing reagent	162-08473	Phenyl Isothiocyanate	for Amino Acid Automated Analysis	1 mL×5	
	166-08476			5 mL×5	
	208-02643	Triethylamine	Wako Special Grade	25 mL	
	202-02641			100 mL	
	056-03341	Ethanol (99.5)	for HPLC	1 L	
	052-03343			3 L	
	088-01805	20% Hydrochloric Acid	Super Special Grade	500 mL	
HPLC column	235-59281	Wakopak® Wakosil®-PTC 4.0*150 mm	-	1 EA (D)	
	231-59283			1 EA (W)	
	232-59291	Wakopak® Wakosil®-PTC 4.0*200 mm		1 EA (D)	
	238-59293			1 EA (W)	
	235-59301	Wakopak® Wakosil®-PTC 4.0*250mm		1 EA (D)	
	231-59303			1 EA (W)	
Eluent	163-14971	PTC-Amino Acids Mobile Phase A	for Analysis of PTC-amino Acids	1 L	
	160-14981	PTC-Amino Acids Mobile Phase B		1 L	
Mixture standard solution	012-28141	Amino Acids Mixture Standard Solution, Type AN (High Range)	for Amino Acid Automated Analysis	1 mL×5A	
	016-28144			5 mL	
	019-28151	Amino Acids Mixture Standard Solution, Type B (High Range)		1 mL×5A	
	013-28154			5 mL	
	016-28161	Amino Acids Mixture Standard Solution, Type H (High Range)		1 mL×5A	
	010-28164			5 mL	
	015-27891	Amino Acids Mixture Standard Solution, Type AN [CRM]		for Amino Acid Automated Analysis	1 mL×5 A
	011-27871	Amino Acids Mixture Standard Solution, Type B [CRM]			1 mL×5 A
018-27881	Amino Acids Mixture Standard Solution, Type H [CRM]	1 mL×5 A			

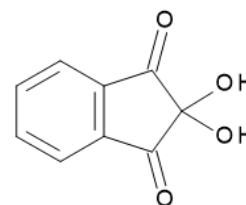
Ninhydrin Color Development Method

Ninhydrin color development method

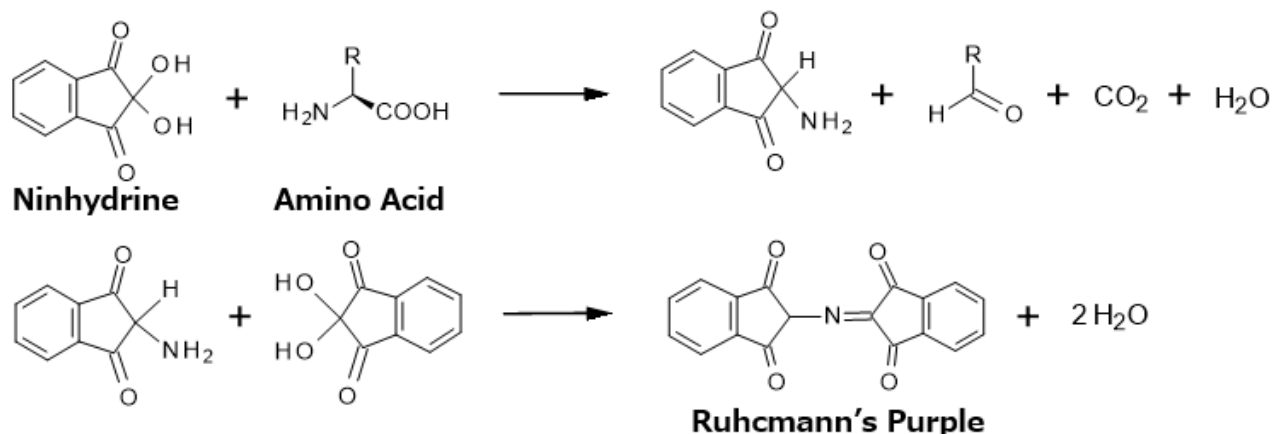
Post-column derivatization method

In the ninhydrin color development method, each amino acid is separated by column chromatography, then reacted with ninhydrin to develop color and absorbance is measured (post-column method).

Ninhydrin color development method is an analytical method with excellent quantification and reproducibility, which is widely used for automated amino acids analysis.



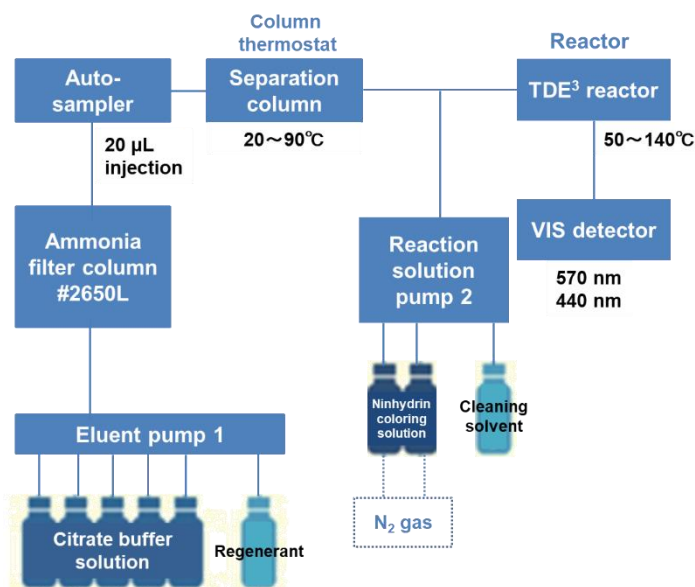
Ninhydrin



Amino acids reacted with ninhydrin reaction reagent are detected using two wavelengths, i.e., 570 nm and 440 nm. The primary amine amino acid (α -amino acid) is colored purple at 570 nm, while the secondary amine (imino acid) amino acid is colored yellow at 440 nm.

Measurement principle

1. The sample is pressured into an alkaline-cation exchange resin. When the eluent pH is increased in a stepwise manner, a higher amount of acidic amino acid is eluted early.
2. When this eluate is heated after the addition of Ninhydrin Coloring Solution, a colored substance showing the local maximum absorption is produced at 570 nm for α -amino acid and 440 nm for imino acid.
3. The peak area of this colored substance is compared with that of the standard solution to determine the concentration of each amino acid in the sample.



Amino acid analyzers corresponding ninhydrin coloring solution

FUJIFILM Wako has ninhydrin coloring solution compatible with Hitachi's and JEOL's amino acid analyzers.



▼ Hitachi High-speed Amino Acid Analyzer: LA8080 Amino SAAYA

Focuses on operability and safety

In addition to the conventional front access design, the reagent-installing unit set at a height for easier operations facilitates routine reagent replacement, sample setting, and maintenance, thereby minimizing manipulations and operations that require a half-sitting posture (the front door can also be removed).

The long-term reliability and stability of the analyzer have been proved

You can still use the same analytical conditions, columns (fillers) and reagents that you have accumulated over the years. In addition, unstable ninhydrin reaction reagents usually need to be refrigerated, but for Hitachi amino acid analyzers, refrigeration is not required, since two solutions are mixed immediately before the derivatization reaction.

The analyzer is compatible with diverse applications

Up to six types of buffer solutions and time program for column thermostat can be used. Moreover, unique features of the reactor, such as high-temperature proof, enable the development of desired methods.

Ninhydrin Coloring Solution Kit for HITACHI

Code No.	Product Name	Grade	Volume
299-70501	Ninhydrin Coloring Solution Kit for HITACHI	for HITACHI High Performance Amino Acids Analyzer	2 L

▼ Amino Acid Automated Analyzer Manufactured by JEOL: JLC-500/V



It provides highly accurate and reproducible data

Highly accurate and reproducible data can be obtained due to technologies, such as vacuum degasser to remove dissolved gas in buffer solution, pump with constant-flow sensor feedback, phase-control pulse mixing type that can efficiently mix ninhydrin and eluent.

It employs a tandem column

It employs a tandem column (multi-stage laminated pressure dispersion column) that prevents the resin from crushing and clogging owing to high pressure and high speed, and this extends the column service life.

It is compatible with GLP

Software installed includes those for equipment performance validation (instrument, analytical method); system suitability test during routine analysis; equipment maintenance; analytical method change; and performance check.

Ninhydrin Coloring Solution Kit for JEOL

Code No.	Product Name	Grade	Volume
295-70601	Ninhydrin Coloring Solution Kit-II for JEOL	for JEOL-Amino Acid Automated Analyzer	2 L

*For inquiries about amino acid analyzers, please contact each device manufacturer.

Ninhydrin Color Development Method

Buffer

In the ninhydrin method, the preparation of the buffer used (e.g., pH, concentration) is very complicated. FUJIFILM Wako offers buffer solutions for exclusive use with type LA-8080, L-8900, L-8800 (A), and L-8500 (A) high-speed amino acid analyzers manufactured by Hitachi High-Tech Corporation.

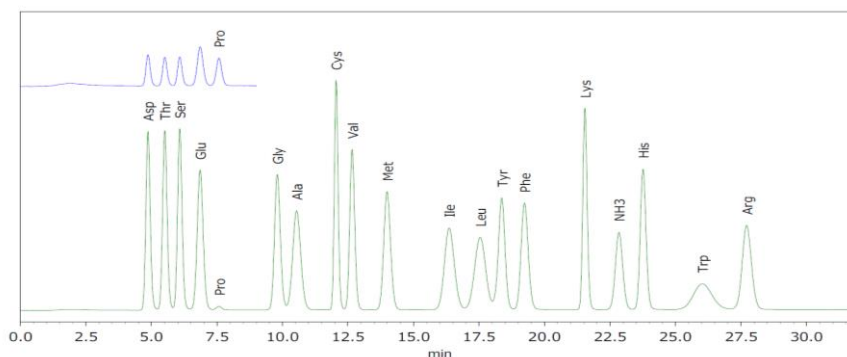
There are two types of analytical methods for ninhydrin color development: “physiological fluid analysis method” and “protein hydrolysate analysis method”.

PF series of buffers are used for the physiological fluid analysis method, and PH series are buffers for the protein hydrolysate analysis method. FUJIFILM Wako also offers set products that include all buffers.

Analytical Method	About	Buffer Series
Protein hydrolysate analysis method	An analytical method targeting 17 constituent amino acids of proteins, peptides, etc.	PF series
Physiological fluid analysis method	An analytical method targeting 41 amino acids contained in physiological fluids such as serum and urine.	PH series

Example of use (PH series)

PH/PF series have a linear baseline and provides good separation of each amino acid.



Buffer Solution for Hitachi High-speed Amino Acid Analyzer

Code No.	Product Name	Grade	Volume
PF series			
025-19521	Buffer for High-Speed Amino Acid Analyzer PF-1	for HITACHI High Performance Amino Acids Analyzer	1 L
022-19531	Buffer for High-Speed Amino Acid Analyzer PF-2		1 L
029-19541	Buffer for High-Speed Amino Acid Analyzer PF-3		1 L
026-19551	Buffer for High-Speed Amino Acid Analyzer PF-4		1 L
023-19561	Buffer for High-Speed Amino Acid Analyzer PF-5		1 L
020-19571	Buffer for High-Speed Amino Acid Analyzer PF-SET		1 set
PH series			
020-19451	Buffer for High-Speed Amino Acid Analyzer PH-1	for HITACHI High Performance Amino Acids Analyzer	1 L
027-19461	Buffer for High-Speed Amino Acid Analyzer PH-2		1 L
024-19471	Buffer for High-Speed Amino Acid Analyzer PH-3		1 L
021-19481	Buffer for High-Speed Amino Acid Analyzer PH-4		1 L
028-19491	Buffer for High-Speed Amino Acid Analyzer PH-RG		1 L
028-19511	Buffer for High-Speed Amino Acid Analyzer PH-SET		1 set

Amino Acid Mixture Standard Solution

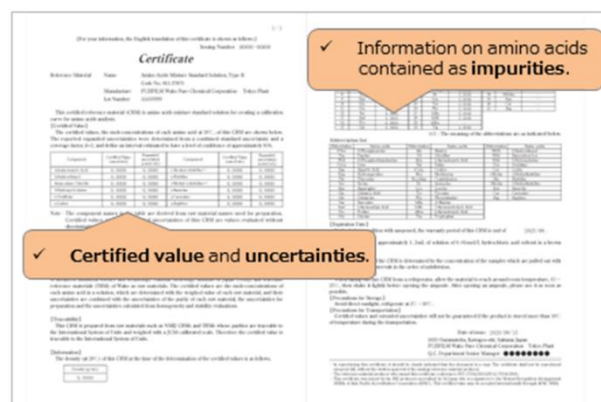
Amino acid mixture standard solution (CRM)

This product is an amino acid mixture standard solution with certified values and uncertainties for each amino acid concentration. It also provides information on amino acids contained as impurities, making it possible to perform accurate amino acid quantification.

These products are certified reference material (CRM) produced under accreditation by the ASNITE Accreditation Program. CRM is attached with a certificate which described metrological traceability, certified values etc.

[Description]

- Certified values and uncertainties for each amino acid
- Density (Reference information)
- Amino acids contained as impurities



Content *Please refer to the CoA for each lot concentration value.

AN Type: 25 mixture standard solution, mainly acidic and neutral amino acids

O-Phosphoserine	0.125 $\mu\text{mol/mL}$	Glycine	0.25 $\mu\text{mol/mL}$	Isoleucine	0.25 $\mu\text{mol/mL}$
Taurine	0.125 $\mu\text{mol/mL}$	Alanine	0.25 $\mu\text{mol/mL}$	Leucine	0.25 $\mu\text{mol/mL}$
O-Phosphoethanolamine	0.125 $\mu\text{mol/mL}$	Citrulline	0.25 $\mu\text{mol/mL}$	Tyrosine	0.25 $\mu\text{mol/mL}$
Urea	5 $\mu\text{mol/mL}$	2-Aminobutyric Acid	0.125 $\mu\text{mol/mL}$	Phenylalanine	0.25 $\mu\text{mol/mL}$
Aspartic Acid	0.25 $\mu\text{mol/mL}$	Valine	0.25 $\mu\text{mol/mL}$	β -Alanine	0.25 $\mu\text{mol/mL}$
Threonine	0.25 $\mu\text{mol/mL}$	Cystine	0.125 $\mu\text{mol/mL}$	3-Aminoisobutyric Acid	0.25 $\mu\text{mol/mL}$
Serine	0.25 $\mu\text{mol/mL}$	Methionine	0.25 $\mu\text{mol/mL}$	Hydroxyproline	0.25 $\mu\text{mol/mL}$
Glutamic Acid	0.25 $\mu\text{mol/mL}$	Cystathionine	0.125 $\mu\text{mol/mL}$	Proline	0.25 $\mu\text{mol/mL}$
Sarcosine	0.625 $\mu\text{mol/mL}$				

B Type: 12 mixture standard solution, mainly basic amino acids

4-Aminobutyric Acid	0.25 $\mu\text{mol/mL}$	Ornithine	0.25 $\mu\text{mol/mL}$	3-Methylhistidine	0.25 $\mu\text{mol/mL}$
2-Aminoethanol	0.25 $\mu\text{mol/mL}$	Lysine	0.25 $\mu\text{mol/mL}$	Anserine	0.25 $\mu\text{mol/mL}$
Ammonium Ion	0.25 $\mu\text{mol/mL}$	1-Methylhistidine	0.25 $\mu\text{mol/mL}$	Carnosine	0.25 $\mu\text{mol/mL}$
5-Hydroxylysine	0.25 $\mu\text{mol/mL}$	Histidine	0.25 $\mu\text{mol/mL}$	Arginine	0.25 $\mu\text{mol/mL}$

H Type: 18 mixture standard solution, mainly protein hydrolyzed amino acids

Aspartic Acid	0.25 $\mu\text{mol/mL}$	Valine	0.25 $\mu\text{mol/mL}$	Phenylalanine	0.25 $\mu\text{mol/mL}$
Threonine	0.25 $\mu\text{mol/mL}$	Cystine	0.25 $\mu\text{mol/mL}$	Ammonium Ion	0.25 $\mu\text{mol/mL}$
Serine	0.25 $\mu\text{mol/mL}$	Methionine	0.25 $\mu\text{mol/mL}$	Lysine	0.25 $\mu\text{mol/mL}$
Glutamic Acid	0.25 $\mu\text{mol/mL}$	Isoleucine	0.25 $\mu\text{mol/mL}$	Histidine	0.25 $\mu\text{mol/mL}$
Glycine	0.25 $\mu\text{mol/mL}$	Leucine	0.25 $\mu\text{mol/mL}$	Arginine	0.25 $\mu\text{mol/mL}$
Alanine	0.25 $\mu\text{mol/mL}$	Tyrosine	0.25 $\mu\text{mol/mL}$	Proline	0.25 $\mu\text{mol/mL}$

APDSTAG® Wako Amino Acids Mixture Standard Solution No. 1

L-Aspartic Acid	0.1800~0.2200 $\mu\text{mol/mL}$	DL-3-Aminoisobutyric Acid	0.1800~0.2200 $\mu\text{mol/mL}$
L-Citrulline	0.900~1.100 $\mu\text{mol/mL}$	5-Hydroxy-DL-lysine	0.1800~0.2200 $\mu\text{mol/mL}$
L-Methionine	0.900~1.100 $\mu\text{mol/mL}$	L-Anserine	0.1800~0.2200 $\mu\text{mol/mL}$
2-Aminoethanol	0.1800~0.2200 $\mu\text{mol/mL}$	Sarcosine	0.1800~0.2200 $\mu\text{mol/mL}$
3-Methyl-L-histidine	0.1800~0.2200 $\mu\text{mol/mL}$	L-Cystine	0.900~1.100 $\mu\text{mol/mL}$
L-Hydroxyproline	0.1800~0.2200 $\mu\text{mol/mL}$	4-Aminobutyric Acid	0.1800~0.2200 $\mu\text{mol/mL}$
L-Glutamic Acid	0.900~1.100 $\mu\text{mol/mL}$	1-Methyl-L-histidine	0.1800~0.2200 $\mu\text{mol/mL}$
DL-2-Aminobutyric Acid	0.4500~0.5500 $\mu\text{mol/mL}$	L-Carnosine	0.1800~0.2200 $\mu\text{mol/mL}$

APDSTAG® Wako Amino Acids Mixture Standard Solution No. 2

Taurine	4.500~5.500 $\mu\text{mol/mL}$	L-Threonine	4.500~5.500 $\mu\text{mol/mL}$	L-Serine	4.500~5.500 $\mu\text{mol/mL}$
Glycine	9.00~11.00 $\mu\text{mol/mL}$	L-Alanine	9.00~11.00 $\mu\text{mol/mL}$	L-Valine	9.00~11.00 $\mu\text{mol/mL}$
L-Isoleucine	4.500~5.500 $\mu\text{mol/mL}$	L-Leucine	4.500~5.500 $\mu\text{mol/mL}$	L-Tyrosine	4.500~5.500 $\mu\text{mol/mL}$
L-Phenylalanine	4.500~5.500 $\mu\text{mol/mL}$	L-Ornithine	2.250~2.750 $\mu\text{mol/mL}$	L-Lysine	4.500~5.500 $\mu\text{mol/mL}$
L-Histidine	4.500~5.500 $\mu\text{mol/mL}$	L-Arginine	2.250~2.750 $\mu\text{mol/mL}$	L-Proline	4.500~5.500 $\mu\text{mol/mL}$

	P-Ser	Tau	PEA	Urea	Asp	Hyp	Thr	Ser	Asn	Glu	Gln	Sar	Aad	Pro	Gly	Ala	Cit	Abu	Val	(Cys) ₂	Met
AN	○	○	○	○	○	○	○	○		○		○		○	○	○	○	○	○	○	○
B																					
H					○		○	○		○				○	○	○			○	○	○
APDS1					○	○				○		○						○	○		○
APDS2		○					○	○						○	○	○			○		

	Hcy (Ala)	Ile	Leu	Tyr	Phe	bAla	bAib	4Abu	Trp	MEA	NH ₄	5Hyl	Orn	Lys	1Mehis	His	3Mehis	Ans	Car	Arg
AN	○	○	○	○	○	○	○													
B							○			○	○	○	○	○	○	○	○	○	○	○
H		○	○	○	○						○			○		○				○
APDS1							○	○		○		○			○		○	○	○	
APDS2		○	○	○	○								○	○		○				○

Abbr.	Amino Acid	Abbr.	Amino Acid	Abbr.	Amino Acid	Abbr.	Amino Acid
P-Ser	<i>O</i> -Phosphoserine	Sar	Sarcosine	Ile	Isoleucine	Orn	Ornithine
Tau	Taurine	Aad	2-Amino adipic Acid	Leu	Leucine	Lys	Lysine
PEA	<i>O</i> -Phosphoethanolamine	Pro	Proline	Tyr	Tyrosine	1Mehis	1-Methylhistidine
Urea	Urea	Gly	Glycine	Phe	Phenylalanine	His	Histidine
Asp	Aspartic Acid	Ala	Alanine	bAla	β -Alanine	3Mehis	3-Methylhistidine
Hyp	Hydroxyproline	Cit	Citrulline	bAib	3-Aminoisobutyric Acid	Ans	Anserine
Thr	Threonine	Abu	2-Aminobutyric Acid	4Abu	4-Aminobutyric Acid	Car	Carnosine
Ser	Serine	Val	Valine	Trp	Tryptophan	Arg	Arginine
Asn	Asparagine	(Cys) ₂	Cystine	MEA	2-Aminoethanol		
Glu	Glutamic Acid	Met	Methionine	NH ₄	Ammonium Ion		
Gln	Glutamine	Hcy(Ala)	Cystathionine	5Hyl	5-Hydroxylysine		

Code No.	Product Name	Grade	Volume
015-27891	Amino Acids Mixture Standard Solution, Type AN [CRM]	for Amino Acid Automated Analysis	1mL×5A
011-27871	Amino Acids Mixture Standard Solution, Type B [CRM]		1mL×5A
018-27881	Amino Acids Mixture Standard Solution, Type H [CRM]		1mL×5A
017-27851	APDSTAG® Wako Amino Acids Mixture Standard Solution No.1 [CRM]		2mL×5A
014-27861	APDSTAG® Wako Amino Acids Mixture Standard Solution No.2 [CRM]		2mL×5A

Amino Acid Mixture Standard Solution

Amino Acid Mixture Standard Solution (High Range)

Amino acid mixture standard solution prepared at high range. It can be used for amino acid analysis of various samples such as food and biological samples.

*This products are not a certified reference material.

AN Type: 25 mixture standard solution, mainly acidic and neutral amino acids

O-Phospho-L-serine	1.19~1.31 $\mu\text{mol/mL}$	L-Valine	2.38~2.62 $\mu\text{mol/mL}$
Taurine	1.19~1.31 $\mu\text{mol/mL}$	L-Cystine	2.38~2.62 $\mu\text{mol/mL}$
O-Phosphoethanolamine	1.19~1.31 $\mu\text{mol/mL}$	L-Methionine	2.38~2.62 $\mu\text{mol/mL}$
Urea	47.5~52.5 $\mu\text{mol/mL}$	L-Cystathionine	1.19~1.31 $\mu\text{mol/mL}$
L-Aspartic Acid	2.38~2.62 $\mu\text{mol/mL}$	L-Isoleucine	2.38~2.62 $\mu\text{mol/mL}$
L-Threonine	2.38~2.62 $\mu\text{mol/mL}$	L-Leucine	2.38~2.62 $\mu\text{mol/mL}$
L-Serine	2.38~2.62 $\mu\text{mol/mL}$	L-Tyrosine	2.38~2.62 $\mu\text{mol/mL}$
L-Glutamic Acid	2.38~2.62 $\mu\text{mol/mL}$	L-Phenylalanine	2.38~2.62 $\mu\text{mol/mL}$
Sarcosine	5.94~6.56 $\mu\text{mol/mL}$	β -Alanine	2.38~2.62 $\mu\text{mol/mL}$
Glycine	2.38~2.62 $\mu\text{mol/mL}$	DL-3-Aminoisobutyric Acid	2.38~2.62 $\mu\text{mol/mL}$
L-Alanine	2.38~2.62 $\mu\text{mol/mL}$	L-Hydroxyproline	2.38~2.62 $\mu\text{mol/mL}$
L-Citrulline	2.38~2.62 $\mu\text{mol/mL}$	L-Proline	2.38~2.62 $\mu\text{mol/mL}$
DL-2-Aminobutyric Acid	1.19~1.31 $\mu\text{mol/mL}$	Cl Ion (reference value)	0.1~0.13 $\mu\text{mol/mL}$

B Type: 12 mixture standard solution, mainly basic amino acids

4-Aminobutyric Acid	2.38~2.62 $\mu\text{mol/mL}$	1-Methyl-L-histidine	2.38~2.62 $\mu\text{mol/mL}$
2-Aminoethanol	2.38~2.62 $\mu\text{mol/mL}$	L-Histidine	2.38~2.62 $\mu\text{mol/mL}$
Ammonium Ion	2.38~2.62 $\mu\text{mol/mL}$	3-Methyl-L-histidine	2.38~2.62 $\mu\text{mol/mL}$
5-Hydroxy-DL-lysine	2.38~2.62 $\mu\text{mol/mL}$	L-Anserine	2.38~2.62 $\mu\text{mol/mL}$
L-Ornithine	2.38~2.62 $\mu\text{mol/mL}$	L-Carnosine	2.38~2.62 $\mu\text{mol/mL}$
L-Lysine	2.38~2.62 $\mu\text{mol/mL}$	L-Arginine	2.38~2.62 $\mu\text{mol/mL}$
Cl Ion (reference value)	0.1~0.13 $\mu\text{mol/mL}$		

H Type: 18 mixture standard solution, mainly protein hydrolyzed amino acids

L-Aspartic Acid	2.38~2.62 $\mu\text{mol/mL}$	L-Isoleucine	2.38~2.62 $\mu\text{mol/mL}$
L-Threonine	2.38~2.62 $\mu\text{mol/mL}$	L-Leucine	2.38~2.62 $\mu\text{mol/mL}$
L-Serine	2.38~2.62 $\mu\text{mol/mL}$	L-Tyrosine	2.38~2.62 $\mu\text{mol/mL}$
L-Glutamic Acid	2.38~2.62 $\mu\text{mol/mL}$	L-Phenylalanine	2.38~2.62 $\mu\text{mol/mL}$
Glycine	2.38~2.62 $\mu\text{mol/mL}$	Ammonium Ion	2.38~2.62 $\mu\text{mol/mL}$
L-Alanine	2.38~2.62 $\mu\text{mol/mL}$	L-Lysine	2.38~2.62 $\mu\text{mol/mL}$
L-Valine	2.38~2.62 $\mu\text{mol/mL}$	L-Histidine	2.38~2.62 $\mu\text{mol/mL}$
L-Cystine	2.38~2.62 $\mu\text{mol/mL}$	L-Arginine	2.38~2.62 $\mu\text{mol/mL}$
L-Methionine	2.38~2.62 $\mu\text{mol/mL}$	L-Proline	2.38~2.62 $\mu\text{mol/mL}$
Cl Ion (reference value)	0.1~0.13 $\mu\text{mol/mL}$		

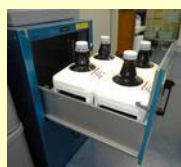
	P-Ser	Tau	PEA	Urea	Asp	Hyp	Thr	Ser	Asn	Glu	Gln	Sar	Aad	Pro	Gly	Ala	Cit	Abu	Val	(Cys) ₂	Met	
AN	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
B																						
H					○		○	○		○				○	○	○			○	○	○	○

	Hcy (Ala)	Ile	Leu	Tyr	Phe	bAla	bAib	4Abu	Trp	MEA	NH ₄	5Hyl	Orn	Lys	1Mehis	His	3Mehis	Ans	Car	Arg	
AN	○	○	○	○	○	○	○														
B								○		○	○	○	○	○	○	○	○	○	○	○	○
H		○	○	○	○						○			○		○					○

Abbr.	Amino Acid	Abbr.	Amino Acid	Abbr.	Amino Acid	Abbr.	Amino Acid
P-Ser	O-Phosphoserine	Sar	Sarcosine	Ile	Isoleucine	Orn	Ornithine
Tau	Taurine	Aad	2-Aminoadipic Acid	Leu	Leucine	Lys	Lysine
PEA	O-Phosphoethanolamine	Pro	Proline	Tyr	Tyrosine	1Mehis	1-Methylhistidine
Urea	Urea	Gly	Glycine	Phe	Phenylalanine	His	Histidine
Asp	Aspartic Acid	Ala	Alanine	bAla	β-Alanine	3Mehis	3-Methylhistidine
Hyp	Hydroxyproline	Cit	Citrulline	bAib	3-Aminoisobutyric Acid	Ans	Anserine
Thr	Threonine	Abu	2-Aminobutyric Acid	4Abu	4-Aminobutyric Acid	Car	Carnosine
Ser	Serine	Val	Valine	Trp	Tryptophan	Arg	Arginine
Asn	Asparagine	(Cys) ₂	Cystine	MEA	2-Aminoethanol		
Glu	Glutamic Acid	Met	Methionine	NH ₄	Ammonium Ion		
Gln	Glutamine	Hcy(Ala)	Cystathionine	5Hyl	5-Hydroxylysine		

Code No.	Product Name	Grade	Volume
016-28161	Amino Acids Mixture Standard Solution, Type AN (High Range)	for Amino Acid Automated Analysis	1 mL×5A
010-28164			5 mL
012-28141	Amino Acids Mixture Standard Solution, Type B (High Range)		1 mL×5A
016-28144			5 mL
019-28151	Amino Acids Mixture Standard Solution, Type H (High Range)		1 mL×5A
013-28154			5 mL

Protective Jacket Dedicated to Gallon Glass Jug "GalloTect™"



GalloTect™ is a protective jacket dedicated to gallon glass jug. FUJIFILM Wako has developed GalloTect™ with the desire to add safety to daily testing and research activities, based on user feedback such as "when lifted the gallon glass jug, it smashed and cracked."

- Carry out solvent resistance test with 11 kinds of solvents!**
 Solvent resistance of body material has been confirmed for a week with various solvents.
 Tested solvents: Water, Methanol, Acetonitrile, Ethanol, Acetone, 2-Propanol, DMF, DMSO, Glycerin, Benzyl Alcohol, 1-Butanol
- High durability!**
 A test of pulling from both ends of the GalloTect™ column revealed that it was durable against a force of 350 N (about 35 kg).
- The lid was opened and closed 200 times to confirm that there is no loosening!**
- All-plastics not requiring waste separation!**
 A resin rivet was adopted for the screw portion. When discarding, a troublesome separation is not necessary!
- Just fit your reagent shelf!**
 We focused on the design and eliminated the bulkiness thoroughly. The conventional reagent shelf can be stored gallon glass jug with GalloTect™.

Code No.	Product Name	Volume
293-36321	GalloTect™	1 EA

Amino Acid Reference Material

Traceable Reference Material (TRM)

TRM series is SI traceable reference material which purity is measured by the method established by NMIJ and FUJIFILM Wako added the uncertainty of homogeneity and stability.

Code No.	Product Name	Grade	Volume
013-25011	β -Alanine Reference Material	TRM	500 mg
019-24871	L-2-Aminoadipic Acid Reference Material		500 mg
012-24861	4-Aminobutyric Acid Reference Material		500 mg
010-25021	DL-2-Aminobutyric Acid Reference Material		500 mg
018-25061	2-Aminoethanol Hydrochloride Reference Material		500 mg
011-25051	DL-3-Aminoisobutyric Acid Monohydrate Reference Material		500 mg
012-25721	L-Anserine Nitrate Reference Material		100 mg
017-24931	L-Asparagine Monohydrate Reference Material		500 mg
035-23111	L-Carnosine Reference Material		500 mg
039-23131	L-Citrulline Reference Material		500 mg
030-23801	L-Cystathionine Reference Material		50 mg
074-06281	L(+)-Glutamine Reference Material		500 mg
088-09921	5-Hydroxy-DL-lysine Monohydrochloride Reference Material		500 mg
084-09881	L-Hydroxyproline Reference Material		500 mg
136-17861	1-Methyl-L-histidine Reference Material		100 mg
139-17851	3-Methyl-L-histidine Reference Material		100 mg
150-03211	L(+)-Ornithine Monohydrochloride Reference Material		500 mg
164-25991	O-Phosphoethanolamine Reference Material		500 mg
169-26041	O-Phospho-L-serine Reference Material		500 mg
197-17331	Sarcosine Reference Material		500 mg
208-19571	Taurine Reference Material	500 mg	
205-19601	L-Tryptophan Reference Material	500 mg	

The traceability system is posted on our website.

[TOP > Analytical Chemistry > Reference Material \(SI Traceable\)](#)

<https://labchem-wako.fujifilm.com/us/category/analysis/referencematerial/index.html>

National Metrology Institute of Japan (NMIJ) Certified Reference Material (CRM)

NMIJ produces CRMs, which is added certified value, needed to determine measured values in chemical metrology, including calibration of analytical instruments and evaluation of analytical methods.

Code No.	NMIJ Code No.	Product Name	Manufacture	Volume
634-22121	NMIJ CRM 6011-a	L-Alanine	National Metrology Institute of Japan	0.5 g
637-25592	NMIJ CRM 3011-a	Ammonium Chloride		25 g
634-22143	NMIJ CRM 6017-b	L-Arginine		0.5 g
635-24091	NMIJ CRM 6027-a	L-Aspartic Acid		500 mg
637-26511	NMIJ CRM 6025-a	L-Cystine		0.5 g
638-24081	NMIJ CRM 6026-a	L-Glutamic Acid		500 mg
631-24071	NMIJ CRM 6022-a	Glycine		500 mg
631-25671	NMIJ CRM 6024-a	L-Histidine		0.5 g
637-20411	NMIJ CRM 6013-a	L-Isoleucine		0.5 g
631-22131	NMIJ CRM 6012-a	L-Leucine		0.5 g
635-22151	NMIJ CRM 6018-a	L-Lysine Monohydrochloride		0.5 g
630-26501	NMIJ CRM 6023-a	L-Methionine		0.5 g
634-20421	NMIJ CRM 6014-a	L-Phenylalanine		0.5 g
634-20661	NMIJ CRM 6016-a	L-Proline		0.5 g
634-25661	NMIJ CRM 6021-a	L-Serine		0.5 g
637-25651	NMIJ CRM 6020-a	L-Threonine		0.5 g
630-25641	NMIJ CRM 6019-a	L-Tyrosine		0.5 g
636-14491	NMIJ CRM 6006-a	Urea		10 g
631-20431	NMIJ CRM 6015-a	L-Valine		0.5 g

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit FUJIFILM Wako Laboratory Chemicals site: <https://labchem-wako.fujifilm.com/> / This leaflet may contain products that cannot be exported to your country due to regulations. / Bulk quote requests for some products are welcomed. Please contact us.

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