

## Amino acid labeling agent for HPLC analysis

# DL-Amino Acid Labeling Kit

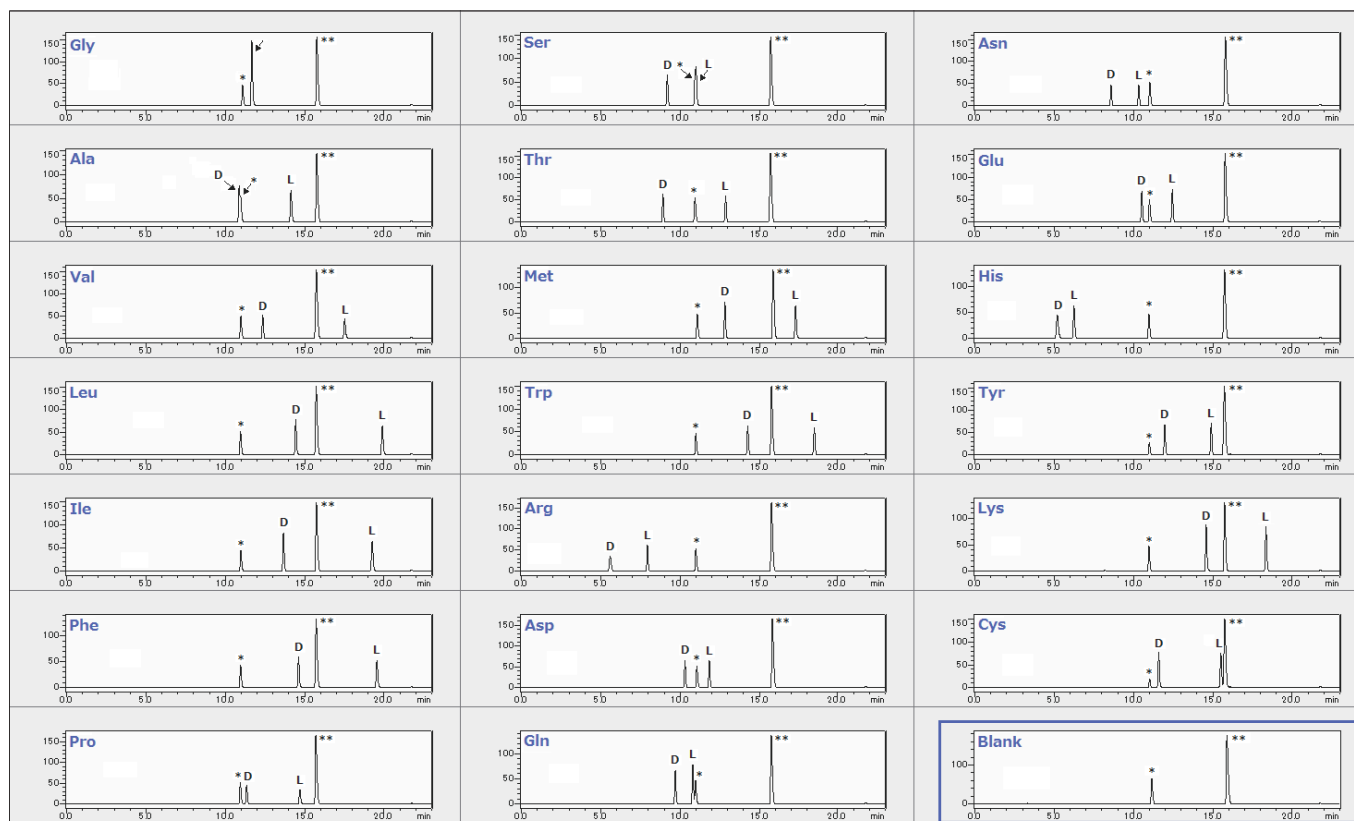
This product is an amino acid labeling kit for HPLC analysis. Due to their high polarity and insufficient UV absorbance, amino acids need to be labeled prior to HPLC. In addition, research on D-amino acids has recently been gaining prominence. With conventional labeling reagents, such as phenyl isothiocyanate and dansyl chloride, costly chiral columns are required. This product is designed to label amino acids without intensive labor and achieve chiral separation with achiral columns, such as C<sub>18</sub>.

- Simple protocol
- Chiral separation with C<sub>18</sub> column
- Compatible with high-sensitivity MS analysis

### HPLC application

#### ■ DL-amino acid separation (protocol 1)

19 DL-amino acids are separable (glycine is not, as it is achiral).



\* (D)FDLDA(Hydrolysed), \*\* (D)DLDA-S-C<sub>6</sub>H<sub>12</sub>OH : Both peaks are derived from the labeling agent itself. See figure 1 on page 2 for details.

#### <Conditions>

Column : COSMOSIL 5C<sub>18</sub>-AR-II 4.6 mm I.D. - 150 mm

Flow rate : 1.0 mL/min

Mobile phase : A: 0.1 % Formic Acid - 20 % Acetonitrile

Temperature : 30°C

B: 0.1 % Formic Acid - 50 % Acetonitrile

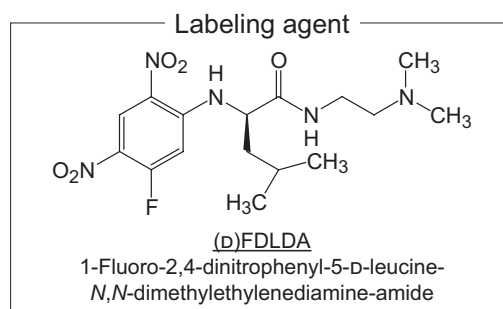
Detection : UV 340 nm

B conc. 0 → 100% 20 min Linear gradient

Sample : (DL)Amino Acid-(D)DLDA derivatives

## Kit components

	Name	PKG size	Required volume / assay
1	Sample* <sup>1</sup>	User-supplied	100 µL
2	Labeling solution* <sup>2</sup>	10 mL	100 µL
3	Initiator solution	10 mL	100 µL
4	Delabeling solution (for side chain)* <sup>3</sup>	10 mL	100 µL
5	Stop solution	10 mL	100 µL
6	Methanol or acetonitrile	User-supplied	500 µL or 600 µL



\*<sup>1</sup> Total amount of functional groups to be reacted should be less than 1.0 µmol. If higher, dilute or reduce sample amount.

\*<sup>2</sup> Uses (D)FDLDA as the labeling reagent.

\*<sup>3</sup> Contains 6-mercapto-1-hexanol [M.W.: 134.24(C<sub>6</sub>H<sub>14</sub>OS)]

## Protocol

### ■ Two protocols are available.

The labeling reagent reacts with amino groups. In addition, some side chains, such as the phenolic hydroxyl group of tyrosine and the thiol group of cysteine, are also labeled. Protocol 1 (step 2) achieves removal of labeling agents on side chains (except for Lys).

**Protocol 1**

**Step 1**  
Add 100 µL each of sample solution, labeling agent solution, and start solution to a glass vessel, seal the vessel, mix in a vortex mixer for 5 seconds, and then react at 50°C for 2 hours.

**Step 2**  
Add 100 µL of the delabeling agent solution for side chain, mix in a vortex mixer for 5 seconds, and then react at 50°C for 15 minutes.

**Step 3**  
Add 100 µL of stop solution and 500 µL of acetonitrile or methanol, then analyze by HPLC (after filtration, if necessary).

**Protocol 2**

**Step 1**  
Add 100 µL each of sample solution, labeling agent solution, and start solution to a glass vessel, seal the vessel, mix in a vortex mixer for 5 seconds, and then react at 50°C for 2 hours.

**Step 2**  
Add 100 µL of stop solution and 600 µL of acetonitrile or methanol, then analyze by HPLC (after filtration, if necessary).

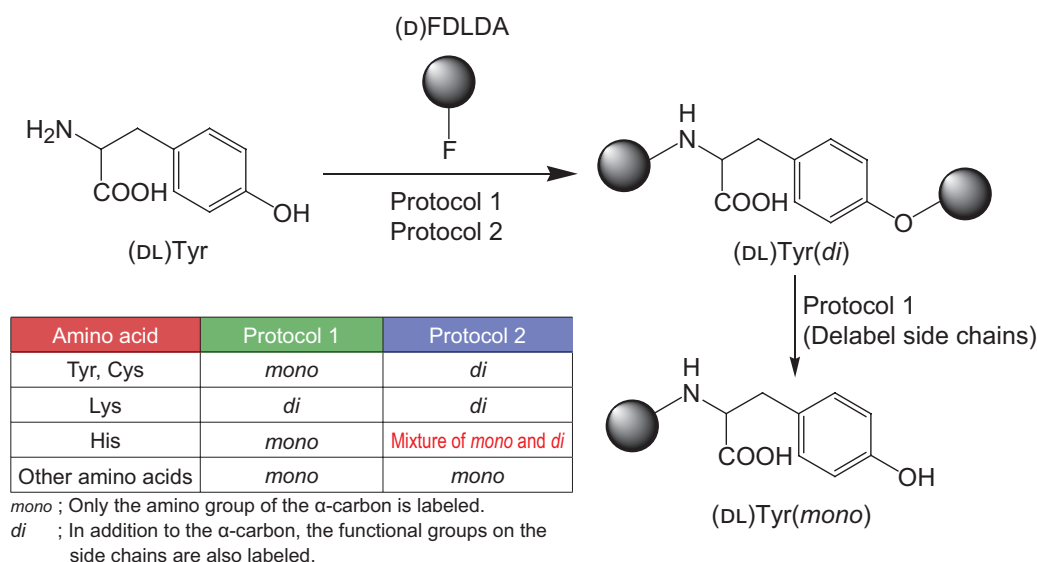


Figure 1: HPLC peaks derived from labeling reagent

Name	Molecular weight	Description
(D)FDLDA	385.39 (C <sub>16</sub> H <sub>24</sub> FN <sub>5</sub> O <sub>5</sub> )	Unreacted labeling reagent (only appears with protocol 2)
(D)FDLDA (Hydrolysed)	383.40 (C <sub>16</sub> H <sub>25</sub> N <sub>5</sub> O <sub>6</sub> )	Hydrolysate of labeling reagent
(D)DLDA-S-C <sub>6</sub> H <sub>12</sub> OH	499.63 (C <sub>22</sub> H <sub>37</sub> N <sub>5</sub> O <sub>6</sub> S)	Reaction product of labeling reagent and delabeling reagent (only appears with protocol 1)

It is strongly recommended that a blank analysis is performed prior to analyzing your sample.

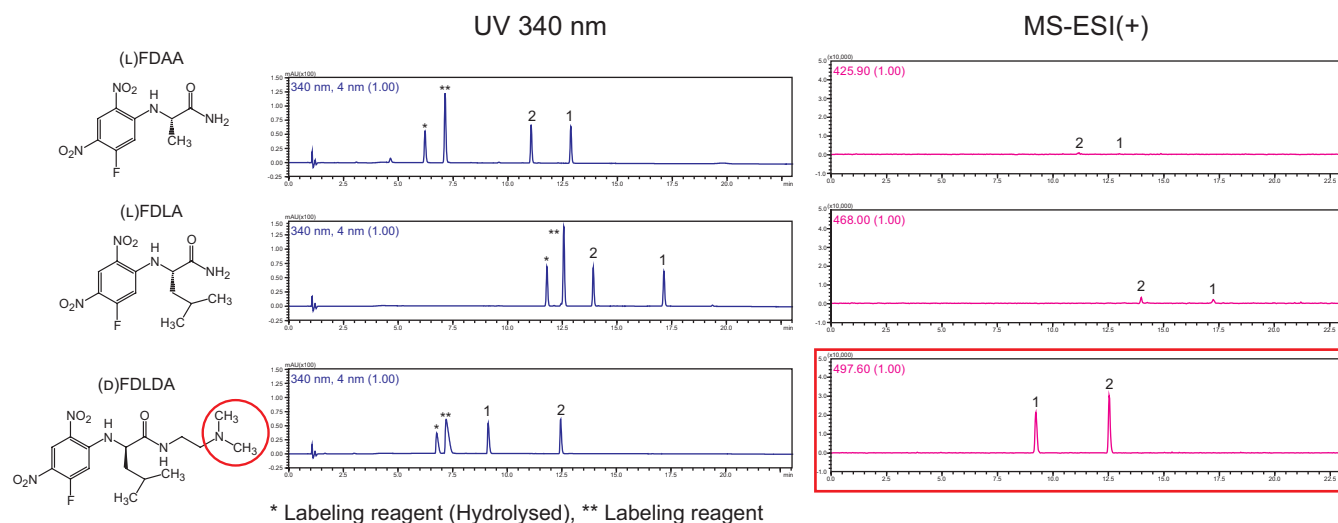
## Reference

Kuranaga, T.; Minote, M.; Morimoto, R.; Pan, C.; Ogawa, H.; Kakeya, H. Highly Sensitive Labeling Reagents for Scarce Natural Products. ACS Chem. Biol. 2020, 15(9), p. 2499-2506.

## LC/MS application

### ■ Comparison with other labeling reagents

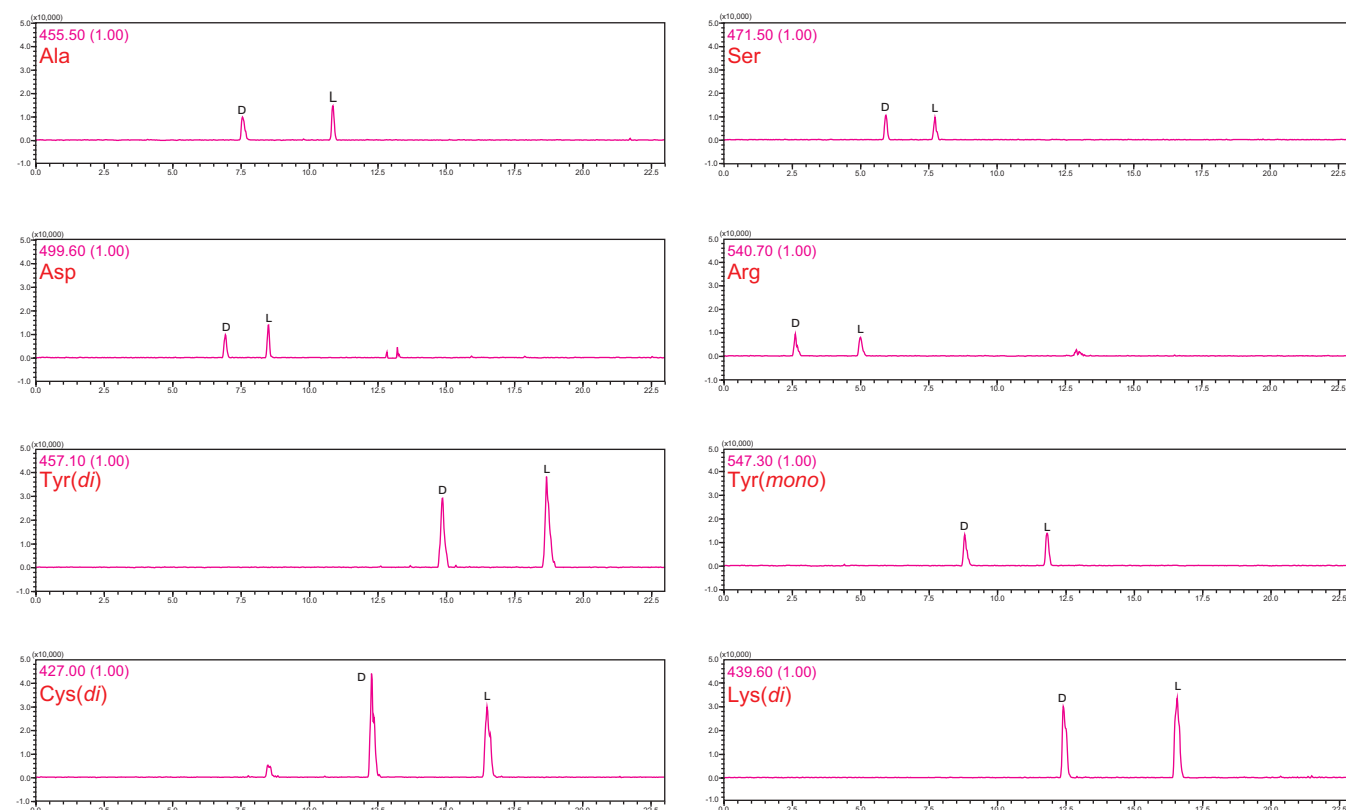
For chiral separation of amino acids by achiral columns, (L)FDAA or (L)FDLA are well-known labeling reagents based on Marfey's method. This kit's labeling reagent (D)FDLDA is easily ionized at the red circle in the structure below, so MS sensitivity is higher compared to the conventional method, while UV sensitivity is about the same. Since the D-form labeling reagent is used, elution order is reversed compared to the conventional method.



<Conditions>

Column	: COSMOCORE 2.6C <sub>18</sub> 2.1 mm I.D. - 100 mm	Flow rate	: 0.2 mL/min
Mobile phase	: A: 0.1 % Formic Acid - 20 % Acetonitrile	Temperature	: 40°C
	: B: 0.1 % Formic Acid - 70 % Acetonitrile	Detection	: UV 340 nm, MS-ESI (+)
	: B conc. 0 → 100% 20 min Linear gradient	Sample	: Labeled Leucine
			1. D form
			2. L form

### ■ DL-amino acid separation (protocol 2)

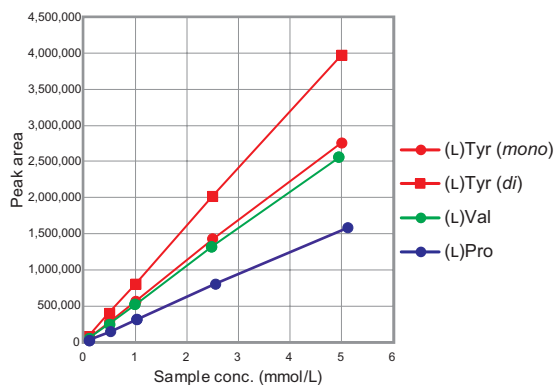


<Conditions>

Column	: COSMOCORE 2.6C <sub>18</sub> 2.1 mm I.D. - 100 mm	Flow rate	: 0.2 mL/min
Mobile phase	: A: 0.1 % Formic Acid - 20 % Acetonitrile	Temperature	: 40°C
	: B: 0.1 % Formic Acid - 50 % Acetonitrile	Detection	: MS-ESI (+)
	: B conc. 0 → 100% 20 min Linear gradient	Sample	: (DL)Amino Acid-(D)DLDA derivatives

## Quantitative performance

Different concentrations of amino acids labeled with this kit were analyzed by HPLC. The calibration curve shows high linearity over the tested concentrations.



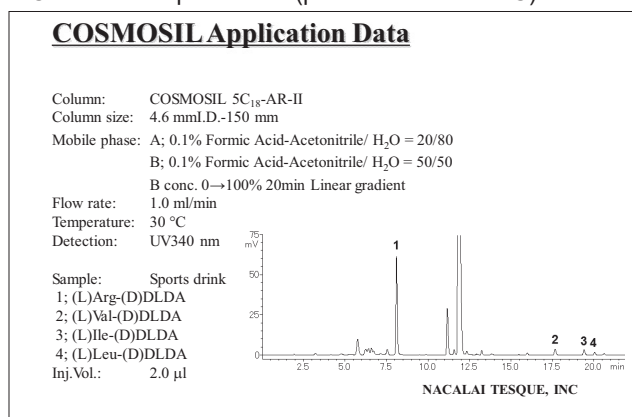
### Labeling reaction

Protocol 1 : (L)Tyr (*mono*)

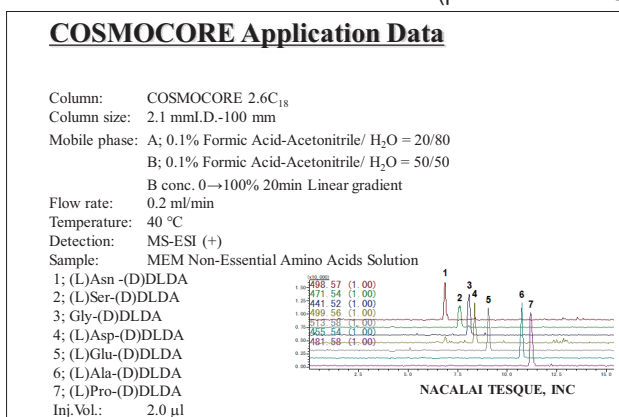
Protocol 2 : (L)Tyr (*di*), (L)Val, (L)Pro

## Other application data

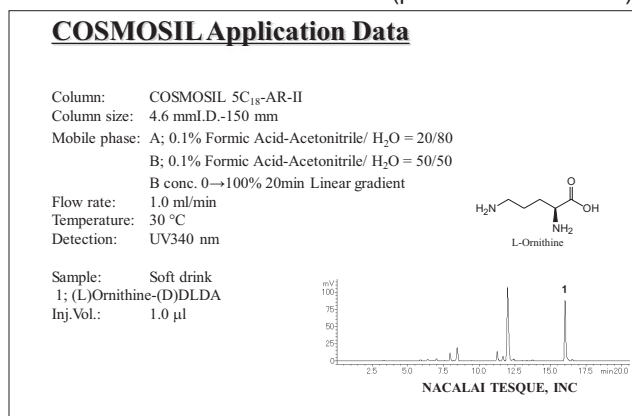
### Commercial sports drink (protocol 2 with HPLC)



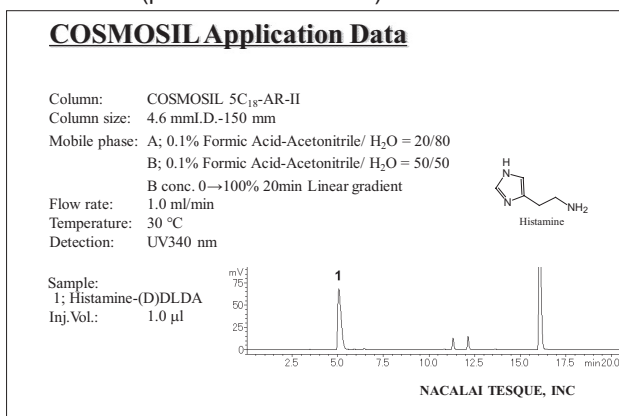
### MEM non-essential amino acids solution (protocol 1 with LC/MS)



### Commercial amino acid soft drink (protocol 2 with HPLC)



### Histamine (protocol 1 with HPLC)



In addition to amino acids, this product is usable for other amine and thiol compounds.

## Ordering information

Product Name	Grade	Storage	Catalog Number	PKG Size
DL-Amino Acid Labeling Kit	SP	Store at 15-25°C	19942-74	100 tests

The labeling reagents contained in the product are patented by Kyoto University. Nacalai Tesque, Inc. produces and distributes this product under license.

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