Amino acid derivatization kit (OPA method, without standards)

Cat NO.: SDK1010

Storage: Stroe at 2-8℃， 1 year.

Size: 25T/50T

Introduction

Derivatization is mainly used to improve the detectability of the target substance and to improve the response of the detector to the target substance. In a weak base solution, OPA and 3-MPA are able to react with the carbonyl and amino groups in amino acids to produce derivatives and emit light longer than the wavelength of the excitation light, called fluorescence, when excited by UV light. The fluorescence intensity of the derivatives is proportional to their concentration, allowing the amount of amino acids in the sample to be calculated. This method has the advantages of rapid determination, high accuracy, high sensitivity and wide range of application.

Special Note: Before starting the experiment, please make sure that the target amino acid to be detected is suitable for the OPA derivatization method.

Apparatus and reagents required for the test

High performance Liquid chromatograph (HPLC), equipped with C18 column (4.6×250 mm, water resistance ≥90%), fluorescence (FLD) detector, adjustable pipette gun, syringe, organic system filter membrane, chromatography grade methanol, chromatography grade acetonitrile and so on.

Product composition (25T/50T):

Product composition		Size(25T)	Size(50T)	Save
Derivative reagents	Reagent I	60 mg×1 bottle, Powder	120 mg×1 bottle, Powder	2-8℃, avoid light
	Reagent II	0.5 mL×1 bottle, Liquid	1 mL×1 bottle, Liquid	RT
	Reagent III	9.5 mL×1 bottle, Liquid	19 mL×1 bottle, Liquid	2-8℃
	Reagent IV	50 μL×1 bottle, Liquid	100 μL×1 bottle, Liquid	RT
Mobile phase	Mobile phase A1	Powder×1 bottle	Powder×2 bottle	RT
	Mobile phase A2	Liquid×1 bottle	Liquid×2 bottle	RT
	Mobile phase A3	Liquid×1 bottle	Liquid×2 bottle	RT
	Mobile phase B	Powder×1 bottle	Powder×2 bottle	RT

Note: 1. All reagents should be stored in airtight condition to prevent volatilization.

2. The product after derivatization is unstable and susceptible to degradation, and needs to be detected in time.

Experimental steps

Pre-experimental preparation

1. Preparation of mobile phase A: Take appropriate amount of ultrapure water to dissolve mobile phases A1, A2 and A3 respectively in the bottle, transfer the dissolved mobile phases A1, A2 and A3 to 1L volumetric flasks, and rinse the walls of the mobile phases A1, A2 and A3 with appropriate amount of ultrapure water for 1-2 times and transfer them to the abovementioned 1L volumetric flasks to ensure that the reagents in the flasks are completely dissolved and moved out, and then finally, the flasks are filled with ultrapure water and volume-determined to 1L. Mix well, pass through a 0.22 μm organic phase membrane and set aside.

2. Preparation of mobile phase B: Take appropriate amount of ultrapure water to dissolve mobile phase B in the bottle, transfer the dissolved mobile phase B to a 1L volumetric flask, and use appropriate amount of ultrapure water to rinse the wall of mobile phase B for 1-2 times and transfer it to the above volumetric flask, and then add 400 mL of chromatographic-grade methanol and 400 mL of chromatographic-grade acetonitrile, and then finally, use ultrapure water to volume the above volumetric flask to 1 L. Mix it well, and pass it through 0.22 μm organic phase membrane before use. Mix well, pass through 0.22 μm organic phase membrane and set aside.

3. Ultrasonic the prepared mobile phases A and B for 30 min to remove the gas in the solvent to prevent blocking the column and affecting the experimental results.

4. Preparation of standard solution (the standard should be prepared by the user): take a certain amount of amino acid standard, dissolve it into 100 μg/ml or 1mM standard solution with 0.1M HCl, and pass it through 0.22 μm aqueous phase membrane.

5. Derivative reagent preparation: take 60 mg of reagent I and add 0.5 mL of reagent II, 4.5 mL of reagent III and 50 μL of reagent IV, mix well, and keep away from light for use. According to this preparation, about 5mL of derivatization reagent can be obtained, which can be used for 25 times of manual derivatization, and the amount of preparation should be increased or decreased according to the specific needs in actual operation.

The steps in the determination

1. Turn on the computer, open the switch button of each module of the Liquid chromatograph (using FLD detector), install the C18 column (4.6×250 mm, water resistance ≥90%), open the software, set the column temperature to 35℃, flow rate to 1 mL/min, excitation wavelength (λex) to 340 nm.

Install the C18 column (4.6×250 mm, water resistance ≥90%), open the software, and set the column temperature at 35℃, flow rate at 1 mL/min, excitation wavelength (λex) at 340 nm, emission wavelength (λem) at 450 nm and excitation wavelength at 1 mL/min.

In the method group, set the column temperature at 35℃, flow rate at 1 mL/min, excitation wavelength (λex) at 340 nm, emission wavelength (λem) at 450 nm, elution program as follows, and the sample walking time at 60 min.

2. Before injection, the column needs to be equilibrated with the initial mobile phase, using the ratio of the initial mobile phase (mobile phase A: mobile phase B = 90:10) to equilibrate the column for 30 min or more, and then start the injection after the baseline is stabilized.

2.1 Automatic derivatization (setting of injection program): Pipette 1 μL of amino acid standard solution, 2 μL of derivatization reagent (pass through 0.22 μm organic phase membrane in advance), 2 μL of reagent III (pass through 0.22 μm aqueous phase membrane in advance), mix in air at maximum mixing speed for 5 times, wait for 1 min, and then inject the sample.

2.2 If the chromatograph does not have automatic derivatization function, manual derivatization can be used: aspirate 100 μL of amino acid standard solution and add 200 μL of derivatization reagent and 200 μL of reagent III, vortex 5 times, each time for 30 s, let stand for 1 min and then pass through the 0.22 μm organic phase membrane, on the sample.

3. The experiment needs to do a blank control at the same time: that is, repeat the above steps with an equal amount of 0.1 M HCl to eliminate interference.

Time（min）	Mobile phase/%	
	Mobile phase A	Mobile phase B
0	90	10
20	65	35
40	20	80
45	20	80
45.10	90	10
60	90	10

Note: 1. Due to the different Liquid phase instruments and column models, the instrument setting conditions can be adjusted according to the actual situation.

2. Derivatization reagents and mobile phases need to be used and prepared now.

3. The product after derivatization is unstable and prone to degradation, and needs to be detected in time.

Note

1. The kit does not contain chromatographic methanol and chromatographic acetonitrile, which should be prepared by yourself.
2. For your safety and health, please wear lab coat and disposable gloves.
3. This reagent is only for scientific research, not for clinical diagnosis or other purposes.
4. If you need customized products, please contact us.