Amino acid derivatization kit (PITC method, without standards)

Cat NO.: SDK1020

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.PITC (Phenyl Isothiocyanate) derivatization is mainly used for the analysis of amino acids, and is based on the principle that the primary amino group of an amino acid reacts with PITC under alkaline conditions. The α-amino group of the amino acid is nucleophilic, while the isothiocyanate group (-N=C=S) in phenyl isothiocyanate is an electrophilic reagent, and the two are capable of undergoing a nucleophilic addition reaction. The reaction first produces an anilino thiocarbonyl (PTC-) amino acid intermediate, which is unstable and will undergo a cyclization reaction under acidic conditions to form a phenylthioacetylurea (PTC-) amino acid. (PTC-) Amino acids have good stability and chromatographic properties, and are very suitable for separation and detection by high performance Liquid chromatography (HPLC) and other analytical methods.

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

Apparatus and reagents required for the test

High performance Liquid chromatograph, C18 column (4.6×250 mm, water resistance ≥90%), chromatography grade acetonitrile, ultrapure water, adjustable pipette gun, syringes, organic and aqueous filtration membranes.

Product composition (25T/50T):

Product composition		Size(25T)	Size(50T)	Save
Derivative reagents	Reagent I	90 μL×1 bottle, Liquid	180 μL×1 bottle Liquid	2-8℃, avoid light
	Reagent II	1.05 mL×1 bottle Liquid	2.1 mL×1 bottle Liquid	RT, avoid light
	Reagent III	14 mL×1 bottle Liquid	28 mL×1 bottle Liquid	RT, avoid light
	Reagent IV	1.25 mL×1 bottle Liquid	2.5 mL×1 bottle Liquid	RT
	Reagent V	25 mL×1 bottle Liquid	50 mL×1 bottle Liquid	RT, avoid light
Mobile phase	Mobile phase A1	Powder×1 bottle	Powder×2 bottle	RT
	Mobile phase A2	Liquid×1 bottle	Liquid×2 bottle	RT, avoid light
	Mobile phase A3	Liquid×1 bottle	Liquid×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 and A2 in the bottle respectively, transfer the dissolved mobile phases A1 and A2 to a 1L volumetric flask, and rinse the walls of mobile phases A1 and A2 with appropriate amount of ultrapure water for 1-2 times and transfer them to the 1L volumetric flask mentioned above 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 mixed to 1L, and then mixed well with mobile phase A3. Adjust the pH of the above solution to 6.2, pass through a 0.22 μm organic phase membrane and wait for use.
2. Preparation of mobile phase B (to be prepared): acetonitrile: water = 4:1 (v/v), mix well, pass through a 0.22 μm organic phase membrane and wait for use.
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 1 mM standard solution with 0.1M HCL, and pass it through 0.22 μm aqueous phase membrane.
5. Preparation of Derivative Reagent 1: Pipette 90 μL of Reagent I and 7.41 mL of Reagent III and mix.
6. Derivative Reagent 2: Pipette 1.05 mL of Reagent II and 6.45 mL of Reagent III and mix well.

Derivative Steps

Pipette 50 μL of standard working solution in a stoppered test tube add 250 μL of Derivative Reagent 1 and 250 μL of Derivative Reagent 2, mix well, and leave for 1 h at room temperature, then add 50 μL of Reagent IV, mix well.

Extraction and purification

To the above derivatized solution, 1 mL of reagent V was added, and the vortex mixer was shaken for 1 min, and after static stratification, the upper layer of solution was discarded, and the lower layer of solution was carefully aspirated by using a needle syringe, filtered through 0.45 μm organic system filter membrane, and used for the determination of liquid chromatography.

The steps in the determination

1. Turn on the computer, open the switch button of each module of the liquid chromatograph (using VWD detector), install the C18 column (4.6×250 mm, water resistance ≥90%), open the software, set the column temperature to 40 ℃, flow rate of 1.0 mL/min, wavelength of 254 nm, injection volume of 10 μL, the elution program as shown in the table below, and the walking time of 50 min. After setting, save the method group.
2. Before injection, the column should be equilibrated with the initial mobile phase, and the column should be equilibrated with the ratio of the initial mobile phase (mobile phase A: mobile phase B = 92:8) for 30 min or more, and the injection should be carried out after the baseline is stabilized.
3. The experiment needs to do a blank control at the same time: i.e., repeat the above steps with an equal amount of 0.1 M HCL to exclude interference.

Time（min）	Mobile phase/%	
	Mobile phase A	Mobile phase B
2	92	8
10	90	10
12	81	19
19	74	26
21	65	35
31	54	46
33	0	100
36	0	100
38	92	8
50	92	8

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 should be prepared on the spot.

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

4. Mobile phase A is a salt mobile phase, after detection, the column should be rinsed with water:acetonitrile=90:10 for 1 h, and then rinsed with water:acetonitrile=10:90 for 30 min or more, so as to avoid the salt residue causing damage to the column.

Note

1. Chromatography grade acetonitrile and ultrapure water (mobile phase B) are not included in the kit and need to be prepared by yourself.
2. For your safety and health, please wear lab coat, disposable gloves and mask.
3. This reagent is only for scientific research, not for clinical diagnosis or other purposes.
4. For customized products, please contact us.