

Product Data Sheet

Product Name: 2,6-Diaminopimelic acid

Cat. No.: GA20387

Chemical Properties

Cas. No. 583-93-7

Formula $C_7H_{14}N_2O_4$

M.Wt 190.2

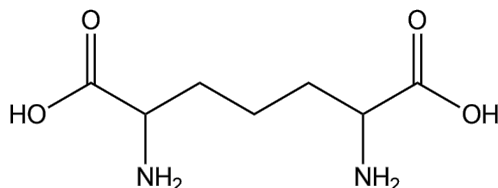
Solubility H_2O : 10.42 mg/mL (54.78 mM;
ultrasonic and warming and heat to
60°C)

Storage Store at -20°C

General For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the
tips ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Evaluation sample solution : ship with blue ice All other available size: ship with RT , or
Condition blue ice upon request.

Structure



Protocol

This protocol is used to distinguish Staphylococci containing 2,6-Diaminopimelic acid (DAP) from Staphylococci containing teichoic acid [1-2]. It is only used as an experimental guide. The actual application needs to be modified according to your needs.

1. Preparation of reagents and materials

Pure culture of Staphylococcus spp. to be tested; DAP standard solution (1mg/mL, dissolved in 0.1M HCl); teichoic acid standard (or a positive control strain known to contain teichoic acid, such as Staphylococcus aureus); 6M hydrochloric acid (HCl), 10% trichloroacetic acid (TCA); ninhydrin colorimetric solution; phosphomolybdic acid staining solution.

2. Experimental steps

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Bacterial cell wall extraction and hydrolysis:

(1) Bacterial collection:

The strain to be tested was inoculated into TSB liquid medium and cultured at 37°C for 18-24 hours. The bacteria were collected by centrifugation (8000rpm, 10min) and washed 3 times with PBS. Freeze-dried cells or directly proceed to the next step of hydrolysis.

(2) Acid hydrolysis (DAP release):

Take 50mg of wet cells, add 1mL of 6M HCl, and hydrolyze at 110°C for 16-18 hours. After cooling, centrifuge (12000rpm, 5min), collect the supernatant, and filter with a 0.22µm filter membrane.

(3) TCA treatment (extract staphylococcal acid):

Take another 50mg of wet cells, add 1mL of 10% TCA, and boil at 100°C for 30min. After centrifugation, take the supernatant for later use.

Thin layer chromatography (TLC) detection:

(1) DAP detection:

Spotting: Spot the acid hydrolyzate and DAP standard on the TLC plate (silica gel G layer).

Developing solvent: n-butanol: glacial acetic acid: water = 4:1:1 (v/v).

Color development: spray ninhydrin solution, bake at 110°C for 5min, and observe purple spots (Rf value of DAP is about 0.3).

(2) Staphylococcal acid detection:

Spotting: TCA extract and positive control are spotted on another TLC plate.

Developing solvent: chloroform: methanol: water = 65:25:4 (v/v).

Color development: spray phosphomolybdic acid staining solution, bake at 105°C for 10min, blue spots indicate the presence of phosphate groups.

Result judgment:

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(1) Staphylococci containing DAP: After TLC color development, the acid hydrolysis sample is consistent with the DAP standard spot position (ninhydrin shows purple), and the staphylococcal acid test is negative.

(2) Staphylococci containing staphylococcal acid: TCA extract shows blue spots (phosphomolybdic acid staining is positive), and the DAP test is negative.

Precautions:

(1) The acid hydrolysis of DAP requires strict temperature control ($110^{\circ}\text{C} \pm 2^{\circ}\text{C}$) to avoid excessive degradation. When extracting staphylococcal acid, TCA treatment for too long may destroy the phosphate group.

(2) It is recommended to combine Gram staining (to confirm bacterial morphology) and biochemical identification (such as catalase test) to assist in differentiation.

(3) If the TLC signal is weak, HPLC or mass spectrometry (LC-MS) can be used for quantitative analysis.

(4) Hydrochloric acid and trichloroacetic acid are corrosive, so gloves and goggles must be worn during operation.

References:

- [1]. Masson HA, Denholm AM, Ling JR. In vivo metabolism of 2, 2'-diaminopimelic acid from gram-positive and gram-negative bacterial cells by ruminal microorganisms and ruminants and its use as a marker of bacterial biomass. Applied and environmental microbiology. 1991 Jun; 57(6): 1714-1720.
- [2]. Bhamidi S, Shi L, Chatterjee D, et al. A bioanalytical method to determine the cell wall composition of Mycobacterium tuberculosis grown in vivo. Analytical biochemistry. 2012 Feb 1; 421(1): 240-249.

Background

2,6-Diaminopimelic acid is a symmetrical α , α' -diamino dicarboxylic acid found in bacteria and higher plants [1]. 2,6-Diaminopimelic acid is a precursor for the biosynthesis of L-lysine and an important component of bacterial peptidoglycan [2]. 2,6-Diaminopimelic acid is a part of the bacteria that changes the bacteria (as a germ of bacteria). Presence or absence of 2,6-Diaminopimelic acid in the cell wall, classification of staphylococcal bacteria (containing 2,6-Diaminopimelic acid) and

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staphylococcal bacteria (containing staphylococcus acid) [3-4].

References:

- [1]. Dzierzbicka K. Synthesis of 2, 6-diaminopimelic acid (DAP) and its analogues. Polish Journal of Chemistry. 2007; 81: 455-473.
- [2]. Shaikh MS, Kale MA, Sharuk K. Discovery of Heterocyclic Analogs of Diaminopimelic Acid as Promising Antibacterial Agents Through Enzyme Targeted Inhibition of Lysine Biosynthesis. Current Enzyme Inhibition. 2018 Aug 1; 14(2): 120-130.
- [3]. Masson HA, Denholm AM, Ling JR. In vivo metabolism of 2, 2'-diaminopimelic acid from gram-positive and gram-negative bacterial cells by ruminal microorganisms and ruminants and its use as a marker of bacterial biomass. Applied and environmental microbiology. 1991 Jun; 57(6): 1714-1720.
- [4]. Bhamidi S, Shi L, Chatterjee D, et al. A bioanalytical method to determine the cell wall composition of Mycobacterium tuberculosis grown in vivo. Analytical biochemistry. 2012 Feb 1; 421(1): 240-249.

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