

Product Data Sheet

Product Name: HADA
Cat. No.: GC50533

Chemical Properties

Cas. No. 2253733-10-5

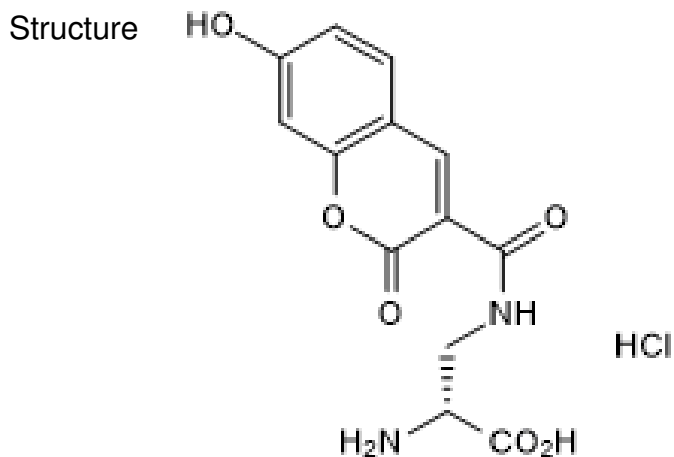
SMILES N[C@@H](C(O)=O)CNC(C1=CC(C=CC(O)=C2)=C2OC1=O)=O.Cl

Formula C₁₃H₁₂N₂O₆.HCl M.Wt 328.71

Solubility DMSO : 125 mg/mL (380.27 mM; Need ultrasonic) Storage Store at -20°C

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

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



Protocol

This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare the following solutions in advance: TSB (or LB), TSB (LB) agar plate, 50 mM HADA stock solution (in DMSO), 10× and 1× sodium citrate buffer, 1× PBS and 3% paraformaldehyde.
2. Thaw the glycerol stock of the bacterial strain on ice. Use an inoculating loop to streak the strain onto TSB agar plates and incubate overnight at 37 °C. Then inoculate 25 ml of TSB (or LB) medium

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Product Data Sheet

with a single bacterial colony and incubate overnight at 37 °C.

3. Dilute the overnight culture to an OD₅₇₈ of 0.1 in TSB (or LB) media and grow at 37 °C until it reaches an exponential OD₅₇₈ of 0.4.

4. The cells were diluted to OD₅₇₈ 0.1 using TSB (or LB) preheated at 37°C, and 500µl was placed in sterile 1.5 ml micro-tube.

5. Add 2.5 µl of 50 mM HADA stock solution to the sample to a final concentration of 250 µM and incubate at 37 °C with shaking for 30 minutes.

6. Add one-tenth the final volume of pre-chilled 10×sodium citrate buffer (50 µl, pH 2.25) to the growing culture.

7. Centrifuge the sample at 16,200 xg for 2 min at 4 °C. The supernatant was discarded and the precipitate was resuspended using 1.5 ml of ice-cold 1× sodium citrate buffer (pH 3.0).

8. Centrifuge the sample at 16,200 xg for 2 minutes at 4 °C. The supernatant was discarded and the precipitate was resuspended using 1.5 ml of ice-cold 1 x PBS (pH 7.4), then the sample was centrifuged again. Repeat the wash step once with ice-cold 1×PBS (pH 7.4). Carefully discard the supernatant without touching the cell precipitate.

9. Fix the cells by resuspending the cell pellet in 12.5 µl 1×PBS and 12.5 µl 3% paraformaldehyde. Mix well with a pipette and store on ice or at 4 °C until microscopic analysis.

10. Microscopic analysis: 3 µl of fixed cells were pipetted into the well of a prepared microscope agarose slide, and a square cover slip was carefully placed on top, pressing gently. Labeled cells were analyzed using an Eclipse Ti microscope (Plan Fluor×100/1.30 Oil Ph3 DLL objective) equipped with a Cool SNAP HQ2CCD camera, using phase contrast and DAPI channels (excitation wavelength 350/50 nm, emission wavelength 460/50 nm). An exposure time of 100 ms was used for phase contrast analysis, and an exposure time of 1 s was used for fluorescence image analysis.

Notes :

1. Before use, filter with a 0.22 µm filter membrane and pre-cool 10× and 1× sodium citrate buffer, 1× PBS and 3% paraformaldehyde solution on ice.

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2. HADA is barely fluorescent at acidic pH or in unbuffered water, and cells imaged at pH above 7.0 show maximum brightness of HADA labeling. The last two wash steps are critical.
3. Microscopic analysis of labeled cells should be performed immediately after fixation.
4. With this protocol, all analyzed cells were fully labeled, showing stronger signals at the septa of contracting cells. Addition of 10×sodium citrate buffer to growing cells, initial washes with 1× sodium citrate buffer, pH 3.0, and rapid processing of samples on ice can improve the removal of unincorporated HADA and lead to reduced background fluorescence signals and improved signal detection.

References:

[1] Peters K, Pazos M, VanNieuwenhze M S, et al. Optimized Protocol for the Incorporation of FDAA (HADA Labeling) for in situ Labeling of Peptidoglycan[J]. Bio-protocol, 2019, 9(15): e3316-e3316.

Background

HADA (HCC-amino-D-alanine hydrochloride) is a blue fluorescent D-amino acid (FDAA), which is suitable for peptides in living bacteria, which stimulates/emitted $\lambda \sim 405/460$ nm. Without affecting the growth rate, FDAA can effectively conduct an external peripheral and diaphragm marking of different bacterial cell groups to display mitochondrial permeability^[1-2].

The D-FDAA probe can marked PG growth sites in a series of different bacteria. FDAA is mixed with PG through DD transpeptidase (penicillin binding protein, PBP) or LD transpeptidase (LDT) (if existence). FDAA can be marked with species in a fast growth process in only 30 seconds, such as E. coli. HADA is capable of reproducible, stable labeling of PG for most bacterial species, usually without extensive optimization^[1-3].

References:

- [1] Botella H, Yang G, Ouerfelli O, et al. Distinct spatiotemporal dynamics of peptidoglycan synthesis between Mycobacterium smegmatis and Mycobacterium tuberculosis[J]. MBio, 2017, 8(5): 10.1128/mbio. 01183-17.
- [2] Peters K, Pazos M, Edoo Z, Hugonnet JE, Martorana AM, Polissi A, VanNieuwenhze MS, Arthur M, Vollmer W. Copper inhibits peptidoglycan LD-transpeptidases suppressing β -lactam resistance due to bypass of penicillin-binding proteins. Proc Natl Acad Sci U S A. 2018 Oct 16;115(42):10786-

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Product Data Sheet

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[3] Mendes S S, Marques J, Mesterházy E, et al. Synergetic antimicrobial activity and mechanism of clotrimazole-linked CO-releasing molecules[J]. ACS bio & med Chem Au, 2022, 2(4): 419-436.

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