VERSION 1

OCT 03, 2019

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.7xahpie

Protocol Citation: Tjusls China 2019. 18 Monitoring in living bacterial cells by UV-Vis spectroscopy. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7xahpie>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Oct 03, 2019

Last Modified: Oct 03, 2019

PROTOCOL integer ID:
28354

18 Monitoring in living bacterial cells by UV-Vis spectroscopy V.1

Tjusls China¹

¹Tianjin University

Tjusls China
Tianjin University

BEFORE START INSTRUCTIONS

Reference:

Ying Ge, Ya-Jun Zhou, Ke-Wu Yang, Yi-Lin Zhang, Yang Xiang and Yue-Juan Zhang. Real-time activity assays of β -lactamases in living bacterial cells: application to the inhibition of antibiotic-resistant E. coli strains. Mol. Biosyst., 2017,13, 2323-2327

- 1 Pipet 5 μ L NDM-28a BL21(DE3) glycerol bacteria into 5ml LB medium, and 2.5 μ L kanamycin is added. Incubate aiming bacterial liquid at 37°C until its OD600 reach 0.5-0.6 then add inducer

IPTG

- 2 Centrifuge bacterial liquid and add phosphate buffer to resuspend bacterial precipitation, then centrifuge again and discard [phosphate buffer](#). Repeat 3 times to wash precipitate.
- 3 Mix bacterial precipitate in phosphate buffer in incubation, and dilute it. OD600 of the bacterial liquid used for next measurement is 0.15.
- 4 [UV-Vis test](#). Test one experimental group together with 3 different controls. Record the absorption value every 300 seconds, 12 times in total.
[\(1\) 95µL bacterial liquid which express target protein, 5µL cefazolin\(final concentration is 150µM\);](#)
(2) 95µL beta-lactamase(final concentration is decided by characteristic of enzyme), 5µL cefazolin(final concentration is 150µM);
(3) 95µL bacterial liquid which is transferred with blank vector, 5µL cefazolin(final concentration is 150µM);
(4) 95µL phosphate buffer, 5µL cefazolin(final concentration is 150µM).
Then plot the UV-vis spectroscopy with time.
- 5 UV-Vis test.
[\(1\) 95µL bacterial liquid which express target protein, 5µL cefazolin\(final concentration is 150µM\);](#)
(2) 95µL bacterial liquid which express target protein, 5µL meropenem(final concentration is 150µM);
(3) 95µL bacterial liquid which express target protein, 5µL faropenem(final concentration is 150µM);
(4) 95µL bacterial liquid which express target protein, 5µL tetracycline(final concentration is 150µM).
Test the UV absorption peak in 273nm(cefazolin), 307nm(meropenem), 300nm(faropenem), 360nm(tetracycline)
- 6 UV-Vis test.
(1) 94 µL bacterial liquid which express target protein, 5µL cefazolin(final concentration is 150µM), µL inhibitor;
(2) 94µL bacterial liquid which express target protein, 5µL cefazolin(final concentration is 150µM), 1µL [inhibitor's solvent \(100% DMSO\)](#);
(3) 94µL phosphate buffer, 5µL cefazolin(final concentration is 150µM), 1µL inhibitor's solvent (100% DMSO);
(4) 94µL phosphate buffer, 5µL cefazolin's solvent, 1µL inhibitor's solvent (100% DMSO).

Test a series of inhibitor's concentration as a gradient and test 5 parallel control. Then calculate the inhibition rate for each concentration as equation 1, and plot IC50 curve.

Equation 1: Inhibition rate% = $100 \times (1 - ([St] - [Si]) / ([St] - [So]))$

[St] = Initial absorption value of antibiotics

[Si] = [Terminated](#) absorption value of antibiotics with the addition of inhibitors

[So] = Terminated absorption value of antibiotics without the addition of inhibitors