



Oct 06, 2019

18 Monitoring in living bacterial cells by UV-Vis spectroscopy V.2

TJUSLS China¹¹Tianjin University

1

Works for me

dx.doi.org/10.17504/protocols.io.7y8hpzw

TJUSLS China
Tianjin University

BEFORE STARTING

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 I](#). Test one experimental group together with 3 different controls. Record the absorption value every 300 seconds, 12 times in total.
 1. 95 μ L bacterial liquid which express target protein, 5 μ L cefazolin(final concentration is 250 μ M);
 2. 95 μ L beta-lactamase(final concentration is decided by characteristic of enzyme), 5 μ L cefazolin(final concentration is 250 μ M);
 3. 95 μ L bacterial liquid which is transferred with blank vector, 5 μ L cefazolin(final concentration is 250 μ M);
 4. 95 μ L phosphate buffer, 5 μ L cefazolin(final concentration is 250 μ M). Then plot the UV-vis spectroscopy with time.
- 5 Establish a system for the determination of viable bacteria.95 μ L bacterial liquid with different induction time and OD value was mixed with 5 μ L cefazolin(final concentration is 250 μ M)to determine the optimal induction time and OD. Record the absorption value every 300 seconds, 24 times in total.
- 6 UV-Vis test II. Test the UV absorption peak in 273nm(cefazolin), 307nm(meropenem), 300nm(faropenem), 360nm(tetracycline)
 1. 95 μ L bacterial liquid which express target protein, 5 μ L cefazolin(final concentration is 250 μ M);
 2. 95 μ L bacterial liquid which express target protein, 5 μ L meropenem(final concentration is 250 μ M);
 3. 95 μ L bacterial liquid which express target protein, 5 μ L faropenem(final concentration is 250 μ M);
 4. 95 μ L bacterial liquid which express target protein, 5 μ L tetracycline(final concentration is 250 μ M).

7 UV-Vis test III.

1. 94µL bacterial liquid which express target protein, 5µL cefazolin (final concentration is 250µM), 1µL inhibitor;
2. 94µL bacterial liquid which express target protein, 5µL cefazolin (final concentration is 250µM), 1µL inhibitor's solvent (100% DMSO);
3. 94µL phosphate buffer, 5µL cefazolin (final concentration is 250µM), 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% = $100 \times \frac{[St] - [Sj]}{[St] - [So]}$

[St] = Initial absorption value of antibiotics

[Sj] = Terminated absorption value of antibiotics with the addition of inhibitors

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



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited