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 We use this protocol and it's working

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

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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 I.](#) 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 250µM);
 2. 95µL beta-lactamase(final concentration is decided by characteristic of enzyme), 5µL cefazolin(final concentration is 250µM);
 3. 95µL bacterial liquid which is transferred with blank vector, 5µL cefazolin(final concentration is 250µM);
 4. 95µL phosphate buffer, 5µL cefazolin(final concentration is 250µM). Then plot the UV-vis spectroscopy with time.
- 5 Establish a system for the determination of viable bacteria. 95µL bacterial liquid with different induction time and OD value was mixed with 5µL cefazolin(final concentration is 250µM) 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. 95µL bacterial liquid which express target protein, 5µL cefazolin(final concentration is 250µM);
 2. 95µL bacterial liquid which express target protein, 5µL meropenem(final concentration is 250µM);
 3. 95µL bacterial liquid which express target protein, 5µL faropenem(final concentration is 250µM);
 4. 95µL bacterial liquid which express target protein, 5µL tetracycline(final concentration is 250µM).
- 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% = $100 * (1 - ([St] - [Si]) / ([St] - [So]))$

[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