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

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Protocol status: Working

We use this protocol and it's working

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Before start

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) 95 μ L bacterial liquid which express target protein, 5 μ L cefazolin(final concentration is 150 μ M);
 - (2) 95 μ L beta-lactamase(final concentration is decided by characteristic of enzyme), 5 μ L cefazolin(final concentration is 150 μ M);
 - (3) 95 μ L bacterial liquid which is transferred with blank vector, 5 μ L cefazolin(final concentration is 150 μ M);
 - (4) 95 μ L phosphate buffer, 5 μ L cefazolin(final concentration is 150 μ M).Then plot the UV-vis spectroscopy with time.
- 5 UV-Vis test.
 - (1) 95 μ L bacterial liquid which express target protein, 5 μ L cefazolin(final concentration is 150 μ M);
 - (2) 95 μ L bacterial liquid which express target protein, 5 μ L meropenem(final concentration is 150 μ M);
 - (3) 95 μ L bacterial liquid which express target protein, 5 μ L faropenem(final concentration is 150 μ M);
 - (4) 95 μ L bacterial liquid which express target protein, 5 μ L tetracycline(final concentration is 150 μ M).Test the UV absorption peak in 273nm(cefazolin), 307nm(meropenem), 300nm(faropenem), 360nm(tetracycline)
- 6 UV-Vis test.
 - (1) 94 μ L bacterial liquid which express target protein, 5 μ L cefazolin(final concentration is 150 μ M), , μ L inhibitor;
 - (2) 94 μ L bacterial liquid which express target protein, 5 μ L cefazolin(final concentration is 150 μ M), 1 μ L inhibitor's solvent (100% DMSO);
 - (3) 94 μ L phosphate buffer, 5 μ L cefazolin(final concentration is 150 μ M), 1 μ L inhibitor's solvent (100% DMSO);
 - (4) 94 μ L phosphate buffer, 5 μ L cefazolin's solvent, 1 μ L 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