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# 18 Monitoring in living bacterial cells by UV-Vis spectroscopy

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1 Works for me dx.doi.org/10.17504/protocols.io.7xahpie

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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 5μL NDM-28a BL21(DE3) glycerol bacteria into 5ml LB medium, and 2.5μL 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*(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