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

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1 Works for me dx.doi.org/10.17504/protocols.io.7y8hpzw

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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 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