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## Small Micro

## **Supporting Information**

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Self-Assembled Magnetic Bead Biosensor for Measuring Bacterial Growth and Antimicrobial Susceptibility Testing

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## Supporting Information for "Self-Assembled Magnetic Bead Biosensor for Measuring Bacterial Growth and Antimicrobial Susceptibility Testing"

To control for false positives we used magnetic beads that were coated with anti-*E. coli* O157 antibodies, and tested the self-assembled AMBR biosensor against a mixed culture of two non-target challenge organisms: *S. aureus* (ATCC 29213) and a non-O157 *E. coli* (ATCC 25922) at a high inoculum, see **Figure S1**. The growth of the target bacteria was detected without false positive results.



**Figure S1.** False positive control experiments performed with self-assembled AMBR biosensor functionalized with anti-*E. coli* O157. The growth of the target bacteria was successfully detected (red points) while the non-target bacteria didn't result in false positive events (gray points).

## Materials and methods

The results were acquired using the following protocol: 1-2 colonies of an overnight culture of desired organism were suspended in CA-MHB (BBL/297963) (1.0 mL) and vortexed. The suspension was diluted (1:10) and grown up to  $OD_{625}$  of 0.08 - 0.1, corresponding to roughly  $10^8$  CFU mL<sup>-1</sup>. The solution was diluted appropriately (0.98 mL), magnetic particles were added (20 µL of Invitrogen/710.04), and the mix was incubated (at 37° C for 10 minutes). Magnetic separation was performed twice with PBS + 0.05% Tween, once with CA-MHB, and finally the beads were resuspended in EEB (1.0 mL of Oxoid CM0989 supplemented with Fluka 80704-5vL). This final solution was pipetted into a hanging drop plate (23 µL per well in 3D Biomatrix plate, pre-soaked in 1% Pluronic F-127 for 1h). The plate was then subjected to a magnetic field gradient that pulled down the magnetic particles (15 min), forming self-assembled AMBR biosensors in each droplet, which were subsequently monitored in a prototype device.