

## Method Sheet 05

# Measuring bacterial growth by microplate-based absorbance assay

### Overview

The cell density of a bacterial culture can be estimated by measuring the absorbance of the culture at wavelengths of around 600 nm. This method sheet explains how to measure the absorbance of a microplate containing bacterial cultures which have been challenged with plant extracts from the *Phytotitre* library.

### Reagents

- 96-well plate containing bacterial cultures challenged with plant extracts and appropriate controls

### Equipment

- Microplate plate reader capable of measuring absorbance in 96-well microplates at a wavelength at 600 nm (ideally, otherwise anywhere between 540 and 650 nm)

### Method

- 1) The absorbance of the plate should be measured immediately after challenging the plate with vehicle control and plant extracts (i.e. at time = 0), then returned to the 37°C incubator to allow the cultures to continue to grow.
- 2) Switch on the microplate reader and any computer attached to it.
- 3) Open the software necessary to operate the instrument and set the wavelength for absorbance measurement to be as close as possible to 600 nm (see notes below for alternative options).
- 4) There is no requirement for a separate reference wavelength (pigment interference is dealt with later during the data analysis stage).
- 5) At various timepoints (see below), retrieve the microplate containing the challenged bacterial cultures from the incubator.
- 6) If there is any moisture on the base of the plate, wipe it with tissue before putting it in the instrument.
- 7) Align the plate so that well A1 is oriented at top left (i.e furthest from you when facing the instrument).
- 8) Remove the lid from the plate and use the software to initiate measurement of absorbance of every well in the plate (see notes regarding sterility below).
- 9) Ensure that the data are saved with a file name that reflects at least the experiment number, plate number, date and time-point.
- 10) Replace the lid and return the plate to the 37°C incubator for further growth.
- 11) The minimum set of timepoints to measure should be 0 hours (straight after challenge, this is necessary to perform a background correction for plant pigment absorbance), and one timepoint after an overnight incubation (e.g. 18 or 24 hours).

- 12) However, it is often helpful to collect data generated from the same experiment by also measuring absorbance of the same plate at 2, 4, 6 and 48 hours, for example.
- 13) If your bacterial strain takes longer to grow (see below) use a timepoint at which you can visibly see clear growth in column 1.
- 14) Once the final measurement has been taken, the plate can be discarded via the appropriate autoclave or disinfection waste stream.
- 15) Retrieve and back up all data relating to the experiment from the instrument using a USB storage device or similar, and switch off.

## Notes

- Bacterial culture density is best estimated at a wavelength of 600 nm, but if this filter is not available in your instrument, wavelengths of anywhere between 540 and 650 nm will also work well in this assay, since these wavelengths are very little absorbed by the LB medium itself, yet still offer a useful measurement of the light scattering caused by bacterial culture turbidity.
- Removing the lid from the plate while reading gives more reliable results as it avoids any potential issues with clearance within the instrument, and also minimises inter-assay variability that may arise from condensation or writing on the plastic lid.
- In our experience, removing the lid briefly does not cause problems with contamination of the plate from air-borne microbes, primarily because they are out-competed by the many millions of bacteria that are already present in the culture.
- *Escherichia coli* cultures will grow well to a measurable density by 18 to 24 hours, but *Micrococcus luteus* cultures may take longer, so read at a timepoint between 24 and 48 hours.

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