

Microplate reader:

Materials:

4titude black 96-well plate round, clear bottom
CLARIOstar® reader (BMG Labtech)
Permeable seal

Steps:

1. Under sterile conditions, load 200 μ L of bacterial culture to a well
2. Repeat this process for two other wells, so that the culture is in triplicates
3. Carefully place a gas-permeable seal over the microplate, making sure that there are no air bubbles and that each permeable circle is directly above the centre of each well.
4. Set up the appropriate temperature in the microplate reader
5. Place the 96-well plate facing up in the reader
6. Set-up the program for the specific culture
 - a. OD600/660:
 - i. Discrete wavelengths, 1
 - ii. Wavelength: 600/660
 - iii. Well scan: spiral average, 5mm diameter
 - b. sfGFP Fluorescence:
 - i. Focal Height: 7.5
 - ii. Gain: 1500
 - iii. Excitation: 485-15
 - iv. Emission 510-20
 - v. Well scan: Matrix scan 3x3 1mm diameter
 - c. mRFP1 Fluorescence
 - i. Focal Height: 7.5
 - ii. Gain: 1651
 - iii. Excitation: 574-15
 - iv. Emission: 618-22
 - v. Well scan: Matrix scan 3x3 1mm diameter
7. Set up the number of cycles and the amount of time between cycles
8. Change the layout of the 96-well plate in the software if appropriate for time-saving purposes

Method adapted from BMG Labtech technicians