

Protocol Plate reader assay - Well Scan

For the detection of the Peptidosomes with encapsulated bacteria, a plate reader assay was performed using the strains **TMB4131** and **TMB3090**. The plate reader used was a "CLARIOstar®" (BMG Labtech). The results were evaluated using the **MARS Data Analysis** (BMG Labtech) program. From an overnight culture, a 1: 500 day culture was grown in LB medium and incubated up to an OD_{600} between 0.2 and 0.6. Subsequently, such a volume was removed from the daily culture and centrifuged at 16,000 g for 5 min. The cells were then resuspended in 500uL Fmoc-FF solution, which resulted in an OD_{600} between 2.0 and 3.0. Peptidosomes with a volume of 15 μ L were generated and transferred into a 24 well plate filled with 500 μ L of LB medium or water.

In addition, wells containing 500 μ L of water or LB medium as well as a sample of the day culture, with the same OD_{600} in LB medium as that of the Fmoc-FF solution, were also introduced. In addition, a sample of lyophilized eGFP (resuspended in water) was measured.

The measurement was carried out using a well-scan mode in a 25x25 or 30x30 matrix.

In the case of strain **TMB4131**, the wavelength range of $\lambda_0 = 455-485$ nm was excited, the emission was measured in the range $\lambda_E = 495-535$ nm. The measured emission range of the strain **TMB3090** was $\lambda_E = 390-590$ nm.

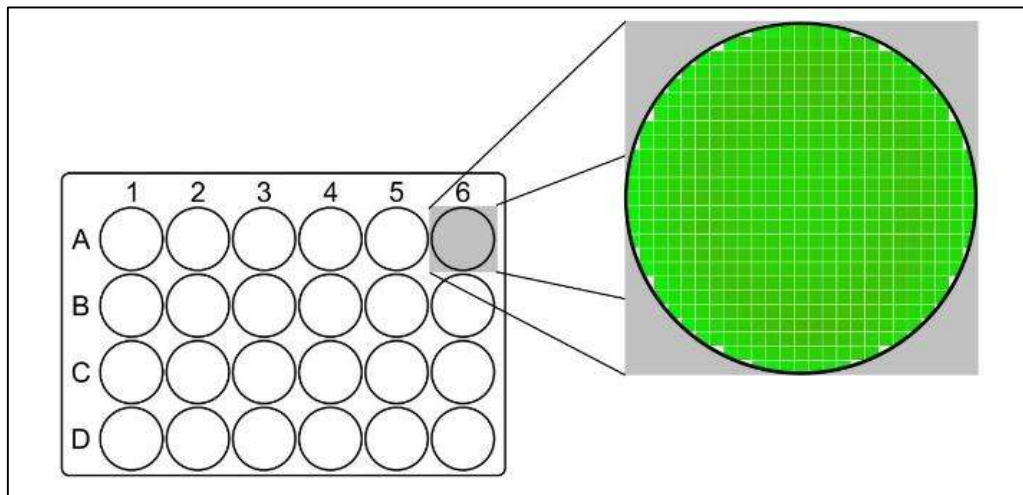


Figure 1: Plate reader assay, well scan mode

Well A6 is shown here as an example for a measurement in which no signal could be detected, which is why the entire matrix is colored green. The presence of a detectable signal would be indicated in red.

For the encapsulation of yeast cells (iGEM Team Aachen collaboration), the strain *Saccharomyces cerevisiae* **BY4741 Δ bar1 Δ far1 GPD-tRFP** was used. This strain secretes the fluorescent protein RFP in a constitutive manner.

Therefore, in this case the wavelength range of $\lambda_0 = 501-532$ nm was excited, whereas the emission was measured in the range $\lambda_E = 600-640$ nm