Test 5

<u>Description</u>: estimation of the evaporation in the plate covered by lid.

Wells filled with: LB+Amp, E0240 diluted from colony in 5ml LB+Amp incubated 1.30 hour 37°C 220 rpm and E0240 picked from colony in 200ul LB+Amp (directly in the multiwell plate)

<u>Purpose</u>: estimation of homogeneity of temperature held inside the instrument. We want to understand the reason why it got contaminated in the previous experiment

Methods: A round-bottom non sterile plate was used. 96 wells were filled with:

- 200 ul LB+Amp,
- 200 ul E0240 from liquid colture, using P1000.
- 200 ul LB+Amp and E0240 colony picked from plate

Protocol:

- The plate was filled as described in Methods
- The instrument temperature was set at 37°C
- After 20 minutes the first Absorbance measurement with lid was performed
- The plate was moved out to remove the lid and a new measurement was performed.
- The plate was covered again with lid and the dynamic cycle of measurement was started:
 - o Duration: 24 hours
 - o Interval: 20 minutes
 - Every cycle: shaking 15 s linear 3mm, waiting 30s and absorbance measurement.

