Protocol for Quantification of bacterial fluorescence using independent calibrants
Protocol code: Quan_fluo

Materials

- Viable *E coli DH5*α
- Liquid medium LB
- Chloramphenicol (60 mg/mL)
- Chamber at 37°C
- 15 flask of 125 mL
- Biotek Synergy HTX Multi-mode microplate reader
- Plasmid DNA (100 pg/uL in 10 uL of Buffer EB)
- Part Device: BBA K225300 in pSB1C3
- Test Device 1: J23101.B0034.E0040.B0015 in pSB1C3
- Test Device 2: J23106.B0034.E0040.B0015 in pSB1C3
- Test Device 3: J23117.B0034.E0040.B0015 in pSB1C3
- Negative Control Device: R0040 in pSB1C3

Procedure

Day 1. Pre culture

1. From a relative fresh plate of each genetic variant of E. coli, pick a colony and growth O/N at 37 °C in a falcon with 25 ml of LB medium and incubate in a shaker.

Day 2. Curve elaboration

1. Centrifuge at 5000 rpm for 10 minutes and resuspended the pellet with LB medium, to a final OD of 0.01.
2. Incubate for 8 h at 37 °C, at slow rpm (120).
3. Take samples every hour.
4. Measure at a wavelength of 600 nm in the Biotek Synergy HTX Multi-mode microplate reader for the OD and measure at 485 nm for excitation and 525 nm for emission to quantity GFP protein.