# 202100302-BioLector AK60

**Strains**

* #385 WT

**AY 1**

**Pre-culture 1:**

* pre-culture in a 96-well plate closed with a gas permeable sealing foil
* Cultures inoculated in 1 ml BHI in a Deepwellplate, from a fresh agar plate
* incubated over day: 30°C, 900 rpm, ~ 8 h

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| A | WT | 1.fs | Ala245fs | WT hrtB-rep | 1.fs hrtB-rep | Ala245fs hrtB-rep |  |
| B | WT | 1.fs | Ala245fs | WT hrtB-rep | 1.fs hrtB-rep | Ala245fs hrtB-rep |  |
| C | WT | 1.fs | Ala245fs | WT hrtB-rep | 1.fs hrtB-rep | Ala245fs hrtB-rep |  |
| D |  |  |  |  |  |  |  |
| E |  |  |  |  |  |  |  |

**Pre-culture 2:**

* 900µl of pre-culture 1 was resuspended in fresh 900 µl CGXII media without iron but PCA
* Incubation over-night: 30°C, 900 rpm, ~ 16 h

**DAY 2**

**Main culture:**

* Measure OD600 of pre-culture 2:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain** | **OD measured** | **Dilution factor** | **OD calculated** | **To pipette µl** | **Well** |
| WT | 0,246 | 100 | 24,6 | 41 | A1, A3, A5 |
| WT | 0,233 | 100 | 23,3 | 43 | B1, B3, B5 |
| WT | 0,225 | 100 | 22,5 | 44 | C1, C3, C5 |
| Ala245fs | 0,227 | 100 | 22,7 | 44 | D1, D3, D5 |
| Ala245fs | 0,261 | 100 | 26,1 | 38 | E1, E3, E5 |
| Ala245fs | 0,233 | 100 | 23,3 | 43 | F1, F3, F5 |
| 1.fs | 0,23 | 100 | 23 | 43 | A2, A4, A6 |
| 1.fs | 0,225 | 100 | 22,5 | 44 | B2, B4, B6 |
| 1.fs | 0,247 | 100 | 24,7 | 40 | C2, C4, C6 |
| Dbind | 0,201 | 100 | 20,1 | 50 | D2, D4, D6 |
| Dbind | 0,235 | 100 | 23,5 | 43 | E2, E4, E6 |
| Dbind | 0,206 | 100 | 20,6 | 49 | F2, F4, F6 |
| DchrS | 0,22 | 100 | 22 | 45 | A7 |
| DchrS | 0,218 | 100 | 21,8 | 46 | B7 |
| DchrS | 0,23 | 100 | 23 | 43 | C7 |
| WT-hrtBrep | 0,202 | 100 | 20,2 | 50 | D7 |
| WT-hrtBrep | 0,213 | 100 | 21,3 | 47 | E7 |
| WT-hrtBrep | 0,208 | 100 | 20,8 | 48 | F7 |
| 1.fs-hrtBrep | 0,195 | 100 | 19,5 | 51 | A8 |
| 1.fs-hrtBrep | 0,178 | 100 | 17,8 | 56 | B8 |
| 1.fs-hrtBrep | 0,203 | 100 | 20,3 | 49 | C8 |
| Ala245fs-hrtBrep | 0,202 | 100 | 20,2 | 50 | D8 |
| Ala245fs-hrtBrep | 0,243 | 100 | 24,3 | 41 | E8 |
| Ala245fs-hrtBrep | 0,194 | 100 | 19,4 | 52 | F8 |

* + Centrifuge: 3 Min 5000 rpm
  + Remove supernatant with pipette
  + Resuspend cells in 1 ml CGXII medium 2% Glucose + 100 µM hemin, except A8-F8, no iron and no PCA in there.
  + 750 µl per well
  + BioLector (Roter Drache) 1200 rpm; 30 °C

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| A | WT  4 µM hemin | 1.fs  4 µM hemin | WT  10 µM hemin | 1.fs  10 µM hemin | WT  20 µM hemin | 1.fs  20 µM hemin | DchrS 20 µM hemin | 1.fs-hrtB rep  4 µM hemin |
| B |
| C |
| D | Ala245fs  4 µM hemin | Δbind  4 µM hemin | Ala245fs  10 µM hemin | Δbind  10 µM hemin | Ala245fs  20 µM hemin | Δbind  20 µM hemin | WT-hrtB rep  4 µM hemin | Ala245fs-hrtB rep  4 µM hemin |
| E |
| F |