

Detection of diacetyl

Materials

LB medium

M9 medium

IPTG

16% TCA

4M HCl

Protocol

- 1、 Inoculate 5 μ L of the BL21- α -ALS-pET-22b(+) into 10mL of the LB which has 10 μ L of Ampicillin(100ug/ul) in it, growing overnight, 30 $^{\circ}$ C, 250rpm.
- 2、 Detect OD₆₀₀ to 1.5-1.6
- 3、 Inoculate 40ul of the culture solution to 20ml M901 medium (M9 medium and 10% LB) of two Eppendorf tubes.(MA &MB). Detect OD₆₀₀ to 0.6-0.8.
- 4、 Add 20ul IPTG into MA, and add nothing into MB. Incubate at 30 $^{\circ}$ C with 250rpm.
- 5、 After 6 hours, add 16% TCA to both MA and MB.
- 6、 Keep on ice for an hour
- 7、 Harvest the supernatant by centrifugation at 8000rpm for 5 min. The supernatant has our wanted diacetyl.
- 8、 Add same amount of TCA to both tubes. Next,add optimum OPD into MA and nothing into MB.
- 9、 Keep in dark for 30 min.
- 10、 Add various aliquot 4M HCl, noting that the amount of HCl of MB is equal to the amount of HCl and IPTG of MA.
- 11、 Detect the concentration of diacetyl using UV spectrophotometry to obtain a series of OD₃₃₅ to make standard curve of diacetyl.