

Carotene Extraction Protocol

1. Take 2 mL cultured yeast solution in 2 mL tube and then centrifuge under 12000 g for 2 min to collect the yeast cells with supernatant dropping out.
2. Add 1 mL ddH₂O to wash the yeast cells and then centrifuge again under 12000 g for 2 min to collect the yeast cells with supernatant dropping out.
3. Dry the tube by air and then weigh the dry yeast cells mass as m_Y .
4. Add 1 mL 6 M HCl to resuspend the yeast cells and then keep in 105 °C metal bath for 3 min to help break the cell wall.
5. Ultrasonic breaking cells procedures
 - 5.a. Take 10 µL yeast cells solution with 90 µL SD-C and 900 µL 1X ConA. (ConA = Concanavalin A from *Canacalia eusiformis*, dilute 2 mg with 9 mL SD-C)
 - 5.b. Ultrasonic break the cells under 135 W in a 0.5 s running and 1 s interval for 8 cycles.
6. Keep the tube quietly on ice for 3 min to cool down and then centrifuge under 12000 g for 2 min to collect the yeast cells with supernatant dropping out.
7. Add 1 mL ddH₂O to wash the yeast cells and then centrifuge under 12000 g for 2 min to collect the yeast cells with supernatant dropping out.
8. Add 1 mL acetone to resuspend the cell debris and vortex to extract the carotene until the cell debris turn to white.
9. Centrifuge the mix under 12000 g for 1 min and draw the acetone extraction solution to a new 1.5 mL tube.
10. Use the spectrophotometer to detect the carotene in the extraction solution and calculate the mass as m_C .
11. Estimate the productivity $Y = \frac{m_C}{m_Y}$.