



YEAST CULTURE PROTOCOL

Pichia Pastoris



OVERVIEW

The heterologous protein expression system of *Pichia pastoris* is now widely used for expression of many human proteins, because the efficiently expressed proteins will be correctly folded in *Pichia pastoris* cells and also efficiently secreted from the cells.

CULTURING PROCEDURE

1. Using a single colony, inoculate 10mL BMGY medium in a 250 mL baffled flask. Grow at 30°C in a shaking incubator (250–300 rpm) until the culture reaches an OD₆₀₀ = 2–6 (log-phase growth, approximately 16–18 hours).
2. Harvest the cells by centrifuging at 1,500–3,000 ×g for 5 minutes at room temperature. Decant the supernatant and resuspend the cell pellet to an OD₆₀₀ of 1.0 in BMMY medium (approximately 50 mL) to induce expression.
3. Place the culture in a 1 liter baffled flask. Cover the flask with 2 layers of sterile gauze or cheesecloth, and return it to the incubator to continue growth.
4. Add 100% methanol to a final concentration of 1% methanol every 24 hours to maintain induction.
5. After three days, collecting bacteria liquid samples, these samples will be used to analyze expression levels. Centrifuge the samples at maximum speed in a tabletop micro centrifuge at room temperature for 2–3 minutes.

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