

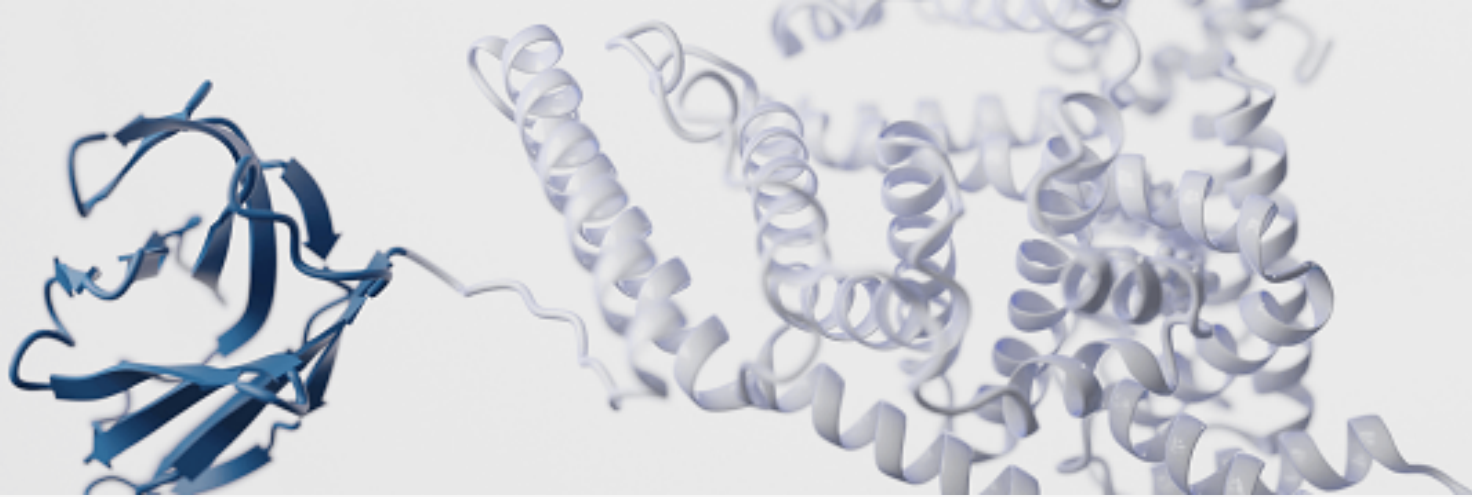


UNLOCK PICHIA®



## METHANOL-FREE PICHIA PASTORIS PROTEIN PRODUCTION TECHNOLOGY

- UNLOCK PICHIA® methanol-free processes leverage engineered AOX1 promoters for high-performance protein expression
- Methanol-free induction preserves the key advantages of the original AOX1 promoter while enhancing process safety
- Glycerol used throughout the process (glucose also possible)
- No metabolic burden from carbon source switching, ensuring smooth and efficient cell growth and protein production
- Short cultivation strategies (~60h) enable process intensification
- Optimized standard cultivation strategies (~110 h) maximize product titers and specific productivity



## **VALIDOGEN's methanol-free system eliminates safety concerns associated with handling methanol while maintaining high expression levels**

We develop high-performance MeOH-inducible and MeOH-free *Pichia* protein production strains for various industries. The MeOH-free system utilizes a specialized subset of our library of AOX1 promoter variants, eliminating the need for methanol as inducer while maintaining the beneficial characteristics of the original AOX1 promoter. Unlike constitutive promoters, this system enables strong, time-controlled initiation of recombinant protein expression. This significantly reduces the metabolic burden associated with change of carbon source, as well as minimizes oxygen consumption and heat evolution. Our methanol-free technology is already in commercial use in bioreactors up to 100,000L.

### **PROCESS INTENSIFICATION**

Recent case studies demonstrate the vast potential of our methanol-free processes for protein expression. In situations where short batch cultivations are advantageous, such as limitations in downstream capabilities, bacterial-like fermentation times in our optimized short culture process (~60h) can deliver high product yields with remarkably high volumetric productivity. In contrast, when maximized batch yield is the ultimate goal our optimized feeding strategies can result in significant titer increases under a standard time cultivation (~110h).

Our MeOH-free AOX1 promoter variants from our UNLOCK PICHIA® toolbox present a unique solution to achieve high protein expression levels in the absence of methanol. These promoters, along with other toolbox elements such as platform strains, helper factors, secretion signals, and expression strategies, work synergistically to enhance strain productivity when applied in optimized combinations.

**Please contact us if you are interested to learn more about VALIDOGEN's methanol-free protein production technology.**

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