



Alagene Pichia Secured™

Methanol-Free Expression Platform

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Technical Brief

Overview

Alagene Pichia Secured™ is a methanol-free recombinant protein expression platform based on a well-established constitutive promoter system in *Pichia pastoris*. The system enables continuous protein expression under standard growth conditions, eliminating the need for methanol induction.

The strain is Generally Recognized As Safe (GRAS) for industrial and food applications

Technical Rationale

Traditional inducible yeast systems rely on methanol to activate expression, introducing:

- Flammability and handling considerations
- Additional process control requirements
- Regulatory and facility compliance complexity
- Induction timing constraints

Alagene Pichia Secured™ replaces induction-dependent control with a constitutive promoter strategy that drives stable expression throughout the cultivation phase while delivering reliable and safe production system.

Key Features

- Constitutive, growth-coupled expression architecture
- Elimination of metabolic transition states
- Simplified feed strategy design
- Improved process predictability
- Reduced oxidative and metabolic stress

Operational Advantages

- Streamlined process development (no induction phase optimization)
- Reduced variability associated with induction timing
- Improved scalability and facility compatibility
- Lower regulatory burden related to hazardous solvent handling

Industry Positioning

Because the constitutive promoter system is broadly adopted and well documented in industry, Alagene Pichia Secured™ supports:

- Straightforward technology transfer
- Established regulatory familiarity
- Predictable scale-up behavior

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Application Scope

Alagene Pichia Secured™ is suitable for:

- Industrial enzymes
- Food and feed proteins
- Therapeutic protein candidates (subject to applicable regulatory frameworks)

Deployment

Alagene Pichia Secured™ is available as part of Alagene's controlled expression platform portfolio under defined service or licensing agreements, ensuring traceability, structured implementation, and clear ownership of resulting developments.

Case study: Comparative analysis of Alagene Pichia Secured™ methanol-free expression performance demonstrated the critical role of screening depth.

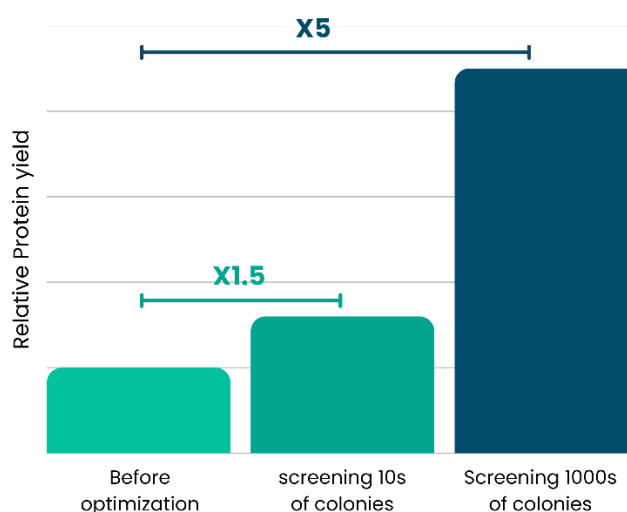


Figure 1. Methanol-free recombinant protein production performance in the Alagene Pichia Secured™ system. Comparative analysis demonstrates the impact of screening scale on expression yield. Limited screening of tens of transformants resulted in an average ~1.5-fold improvement over baseline, whereas high-throughput screening of thousands of colonies enabled identification of superior clones with an approximately 5-fold increase in yield. The data highlight the importance of large-scale clone screening to fully leverage the potential of constitutive, methanol-free expression platforms.