



Scalable Production of High-Purity Nanobodies Using Yeast Expression System

Overview

The increasing demand for advanced biotherapeutics and diagnostics has accelerated the need for efficient and scalable antibody-based technologies. Among these, nanobodies—single-domain antibodies derived from camelids—offer distinct advantages due to their small size, high stability, and exceptional specificity. Their unique structure, compared to conventional antibodies (Figure 1), makes them valuable for therapeutic, diagnostic, and research applications.

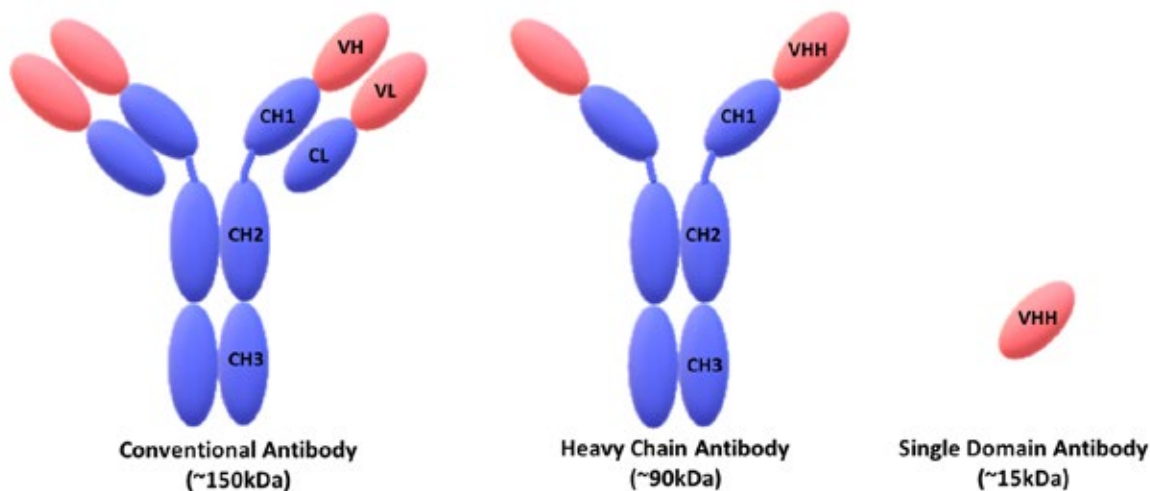


Figure 1: Comparison of the VHH nanobody and a conventional antibody. The VHH nanobody is depicted with its unique single-domain structure, while the conventional antibody is shown with both heavy and light chains.

Challenge

One of the primary challenges in nanobody production is scaling up while maintaining their functionality. The ideal expression system must ensure high yields, correct post-translational modifications, and cost-effective scalability. Among various expression platforms, the yeast expression system with *Komagataella pastoris* [earlier known as *Pichia pastoris* a methylotrophic yeast, has emerged as a superior system for recombinant nanobody production. With extensive expertise in recombinant protein expression, Aragen established *K. pastoris* expression platform for nanobody production, ensuring robust and scalable outcomes.

Aragen's Solution

To establish a high-efficiency *K. pastoris* expression system, Aragen implemented a strategic approach:

- **Optimized Vector Design:** The nanobody gene was cloned into the pPICZα expression vector under the methanol-inducible AOX1 promoter, ensuring controlled expression.
- **Stable Strain Selection:** Recombinant vectors were introduced into *K. pastoris* strain X-33, followed by zeocin-based selection to confirm successful gene integration.
- **Optimized Expression Conditions:** Shake flask cultures were optimized for methanol-induced nanobody secretion over 72 hours, ensuring maximal yield.
- **Efficient Purification:** A two-step purification process was employed:
 - Nickel-Nitrilotriacetic Acid (Ni-NTA) affinity chromatography
 - Size Exclusion Chromatography (SEC)
- **Comprehensive Characterization:** Purified nanobodies were analyzed by SDS-PAGE, analytical Size Exclusion Chromatography (aSEC), and Mass Spectrometry (MS) to confirm purity, molecular weight, and structural integrity.

Outcomes

Aragen successfully established the *K. pastoris* expression system for scalable nanobody production:

- **High Yield:** Achieved 128.25 mg/L purified nanobody yield.
- **Exceptional Purity:** SDS-PAGE and aSEC confirmed 100% purity (Figure 2).
- **Precise Molecular Integrity:** Mass Spectrometry validated the observed molecular weight (14,970.037 Da) against theoretical expectations (Figure 3).
- **Efficient Scalability:** The established system ensures seamless scalability from laboratory research to industrial production, making *K. pastoris* a preferred platform for nanobody manufacturing.

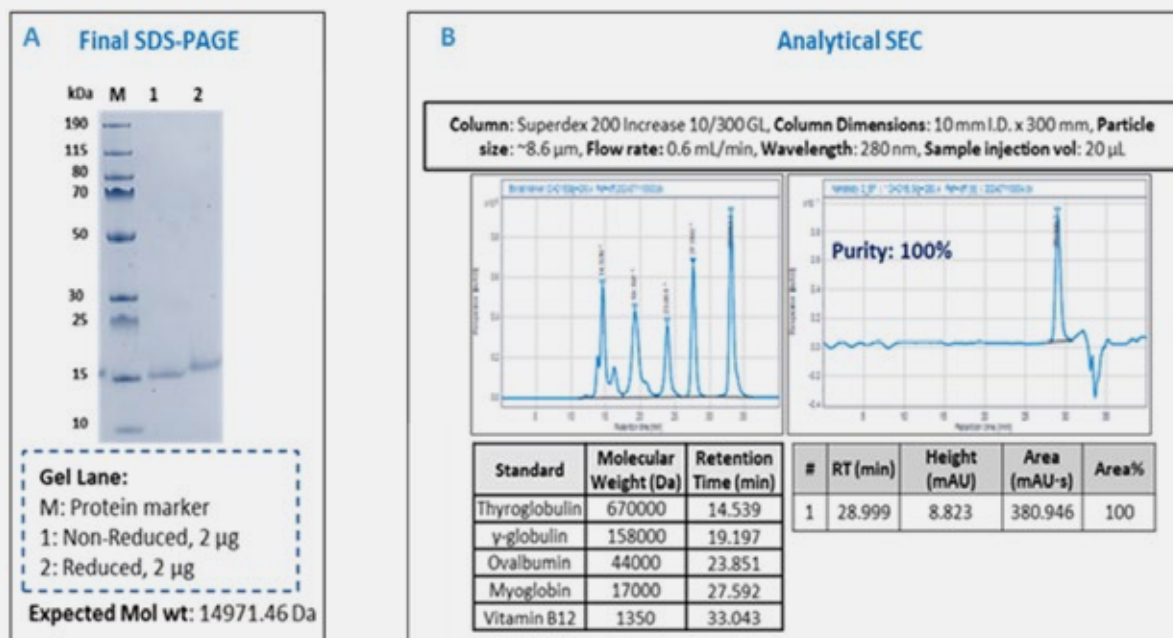


Figure 2: Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) results showing single nanobody bands in lane 1 and 2 (A); and analytical Size-Exclusion Chromatography (aSEC) (B) for the standards (left chromatogram) and the test nanobody (right chromatogram).

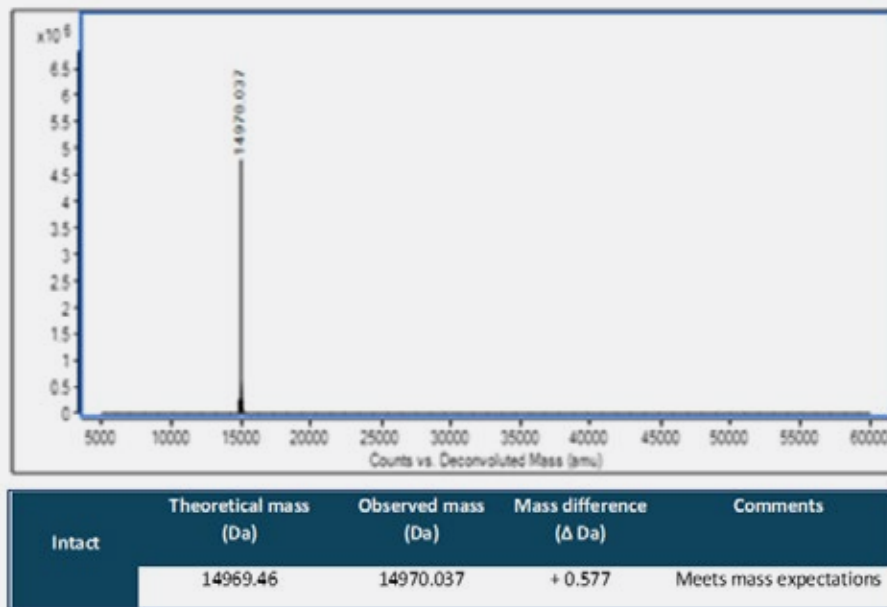


Figure 3: Mass Spectrometry (MS) analysis for the purified target protein which appeared as an individual peak of 14970.037 Da.

Why Aragen?

With over a decade of experience in recombinant protein expression, Aragen is a trusted partner for biopharmaceutical innovation. Our capabilities ensure streamlined protein production and accelerated R&D timelines:

- **Customizable Expression Platforms:** Expertise in bacterial, yeast, baculovirus/insect, and mammalian expression systems tailored to specific project needs.
- **End-to-End Solutions:** Integrated workflows for expression, screening, purification, and characterization, optimizing time and cost.
- **Proven R&D Impact:** Trusted by global biopharmaceutical companies to produce high-quality antibodies, cytokines, and enzymes for therapeutic and diagnostic applications.

Aragen's technical excellence and flexible platforms make it the ideal partner for advancing nanobody-based biotherapeutics and diagnostics.

Let's begin the
Conversation

E: bd@aragen.com

W: aragen.com

[in /company/aragen-life-sciences](https://www.linkedin.com/company/aragen-life-sciences)

[f /AragenLifeSciences](https://www.facebook.com/AragenLifeSciences)



India • USA • Netherlands • Japan • S Korea