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Successful high-throughput expression of 50 VHHs and minibinders from linear DNA template using our SYN-XPRESS™ GOLD VHH KIT

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Traditional cell-based expression systems can be time-consuming and poorly suited for early-stage screening of large VHH libraries. Cell-free protein synthesis (CFPS) offers a powerful alternative, allowing DNA-to-protein synthesis within hours, precise control of reaction conditions, and easy scalability.
The Syn-Xpress™ Gold VHH kit is specifically optimized for the expression of VHHs and other small binding proteins, supporting proper folding and disulfide bond formation. This makes it particularly well suited for high-throughput expression of minibinders and nanobodies.
In this application note, we showcase the performance and robustness of the Syn-Xpress™ Gold VHH kit for the rapid expression and purification of 50 VHHs and minibinders from Twist Biosciences linear DNA in just 24 hours. Even the most challenging constructs were successfully produced and purified using a single-step affinity purification followed by desalting. Purified protein yields ranged from 0.1 to 0.6 mg/mL, with an average purity exceeding 80%, highlighting the kit as a powerful solution for fast, reliable screening of VHHs and minibinders.

INTRODUCTION

Variable domains of heavy-chain antibodies (VHHs), also known as nanobodies, represent a highly attractive class of biomolecules for applications in research and biotechnologies for diagnostics or therapeutic development. Their small size, high stability, and ability to recognize challenging epitopes make them powerful tools.
Cell-free protein expression systems offer a compelling alternative to conventional cell-based expression platforms by enabling rapid protein synthesis, experimental flexibility, and straightforward scalability. These systems are particularly well suited for high-throughput screening, as they allow the parallel expression of multiple DNA constructs without the constraints associated with cell viability or culture optimization.
In this application note, we report the simultaneous expression of 50 distinct VHHs or minibinders using the Syn-Xpress™ Gold VHH kit, a cell-free expression system specifically optimized for nanobody production. Expression performance was evaluated in terms of yield and protein quality, demonstrating the robustness and efficiency of this approach for rapid VHH screening and functional characterization.

Figure 1 – Workflow for VHH production in a Cell-Free System with Syn-Xpress™ GOLD VHH kits : from linear DNA synthesize to protein quantification

MATERIAL AND METHODS

-Syn-Xpress™ GOLD VHH kit (ref SYN90022)
-DNA templates
-96-well plates (nuclease-free)
-Breathable sealing film - Dutscher (#676051)
-Incubator with agitation – Infors (Multitron)
-His MultiTrap TALON – Cytiva (#2900596)
-Zeba Spin Desalt Plate 7kDa – Thermofisher (#89808)
-50mM Tris pH 7.4, 500mM NaCl
-50mM Tris pH 7.4, 500mM NaCl, 5mM Imidazole
-50mM Tris pH 7.4, 500mM NaCl, 30mM Imidazole
-50mM Tris pH 7.4, 500mM NaCl, 150mM Imidazole
-Plate reader for 280nm absorbance

1 Linear DNA preparation

Each VHH or minibinder coding sequence was codon-optimized for *E. coli* to maximize translational efficiency. The sequences were assembled as linear DNA templates incorporating all regulatory elements required for efficient CFPS expression, in accordance with our recommended design guidelines (see Guidelines 2025_DNA Preparation CF kit Guidelines_Synthelis Biotech).
At the 5' end (N-terminal region), a non-coding nucleotide extension was added to enhance linear DNA stability by protecting the template from residual nuclease activity present in the cell-free lysate. This extension was followed by a T7 promoter and a ribosome binding site (RBS) to ensure robust transcription and efficient translation initiation.
The VHH or minibinder coding sequence was positioned downstream of these regulatory elements. They were designed with a S/G linker followed by a 6-histidine tag in C-terminal for protein purification.
At the 3' end (C-terminal region), a T7 transcription terminator was included to ensure proper transcription termination. A second non-coding nucleotide extension was added downstream of the terminator to further improve template stability throughout the CFPS reaction.
Linear DNA templates were synthesized by Twist Biosciences.

5' : +148pb | linker | T7 prom | linker | 5' UTR | linker | codon start (Met)
CGAAACAAGCGCTCATGAGCCCGAAGTGGCGAGCCCGATCTTCCCCATCGGT
GATGTCGGCGATATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCG
GCCACGATGCGTCCGGCGTAGAGGATCGAGATCTCGATCCCGCGAAATTAATA
CGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATTTTGTTTAAC
TTTAAGAAGGAGATATACC (ATG …

3' : codon stop (x2) | linker | T7 term | linker | +138pb
…TAATAA)CTCGAGCGAGCTCTGCAGCCCGGGATCCGGTAACTAACTAAGATCC
GGTAAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGC
CACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGA
GGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGATATCCACAGGACGGGTGT
GGTCGCCATGATCGCGTAGTCGATAGTGGCTCCAAGTAGCGAAGCGAGCAGG
ACTGGGCGGCGGCCAAAGCGGTCGGACAGTGCTCCGAGAACGGGTGCGCAT
AG

Figure 2 – Examples of extremities to prepare linear DNA templates for protein expression in CFPS Syn-Xpress™ kits

2 CFPS reaction

Cell-free protein synthesis reactions were assembled in accordance with the Syn-Xpress™ Gold VHH kit instructions. All reaction components and consumables were maintained on ice and in a nuclease-free environment prior to assembly in order to preserve reagent activity.
Reactions were prepared in a nuclease-free 96-well plate. For each construct, 5 µL of linear DNA template, diluted in nuclease-free water to 100 nM, was dispensed into each well. The CFPS reaction was initiated by the addition of 45 µL of Syn-Xpress™ Gold VHH mastermix, resulting in a final reaction volume of 50 µL per well with 10 nM of linear DNA.
After assembly, the plate was sealed with a breathable sealing film to ensure adequate oxygen exchange while minimizing evaporation during incubation. The reactions were incubated at 25 °C for 16 hours under agitation (200 rpm) to promote efficient protein synthesis and proper folding.
At the end of the incubation period, CFPS reactions were directly used for downstream analyses without further processing.

3 Purification

The supernatants were then loaded onto an His MultiTrap™ TALON® prepacked plate (Cytiva) pre-equilibrated 3 times with nuclease free-water, then 3 times with 50 mM Tris pH 7.4, 500 mM NaCl and 5 mM Imidazole. Binding of His-tagged VHHs or minibinders was performed under native conditions.
After sample loading, the wells were washed 3 times with 50 mM Tris pH 7.4, 500 mM NaCl and 30 mM Imidazole to remove unbound proteins and cell-free system components. Bound proteins were subsequently eluted using 50 mM Tris pH 7.4, 500 mM NaCl and 150 mM Imidazole.
The eluted fractions were immediately subjected to buffer exchange and desalting using a Zeba™ Desalting Plate (Thermo Fisher Scientific), following the supplier's recommendations. This step efficiently removed imidazole and low-molecular-weight contaminants while transferring the proteins into 50 mM Tris pH 7.4, 500 mM NaCl buffer compatible with downstream functional and biophysical assays.
Purified VHHs and minibinders were recovered in a plate format suitable for parallel processing and were directly used for quality control.

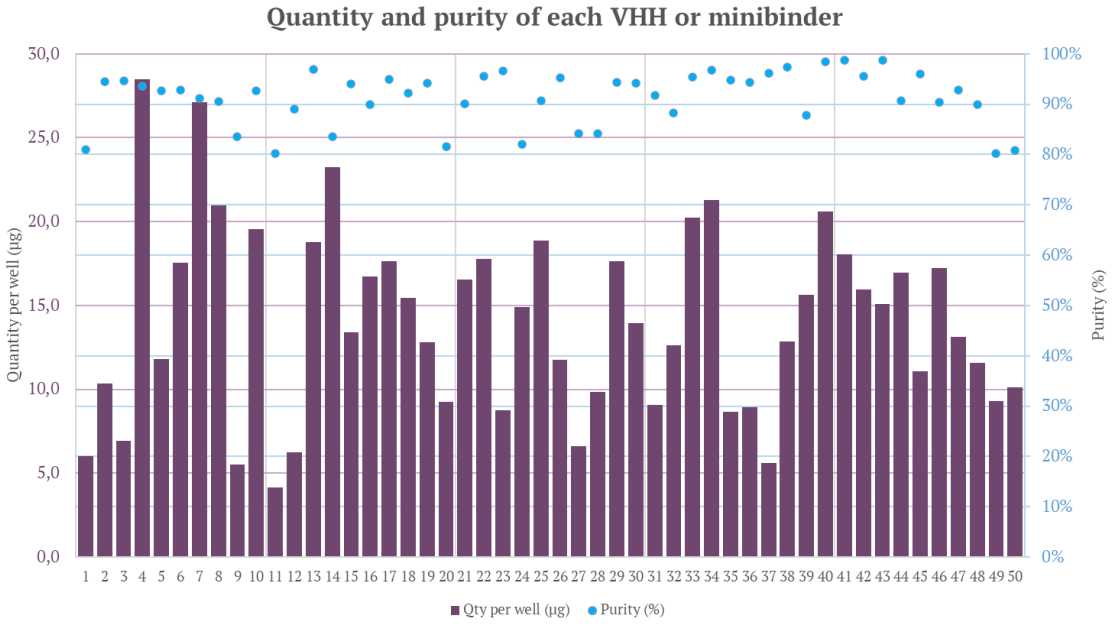
4 Analysis

Protein concentration of purified VHHs and minibinders was first determined by measuring absorbance at 280 nm (A280) using a microplate reader. Measurements were performed directly in plate format using 50 mM Tris pH 7.4, 500 mM NaCl buffer as blank, allowing rapid and parallel quantification of all samples.
Purity and apparent molecular weight of the expressed proteins were evaluated by SDS-PAGE. Samples were diluted in Laemmli sample buffer and denatured by heating at 90 °C for 5 minutes. A volume of 11.25 µL of each protein sample was loaded onto the gels. Following electrophoresis, proteins were visualized by Coomassie Brilliant Blue staining.

RESULTS AND DISCUSSION

Successful Expression of 50 VHHs and Minibinders

Using the Syn-Xpress™ Gold VHH CFPS kit, all 50 VHHs and minibinders constructs were successfully expressed in parallel (Figure 3), including variants considered difficult-to-express due to atypical sequence features. Recovered purified protein amounts ranged from 4.2 µg to 28.5 µg per construct, corresponding to yields of 0.1–0.6 µg/µL, demonstrating the efficiency of the Syn-Xpress™ Gold VHH CFPS kit, which is specially optimized for the expression of VHHs and minibinders, and their correct folding and disulfide bond formation (Graph 1). This targeted optimization resulted in a high expression success rate across all tested constructs, including the most challenging ones, and minimized the need for extensive condition screening typically.



Graph 1 – Quantity determined by absorbance measure at 280 nm and purity determined by SDS-PAGE analysis of the 50 VHH and minibinder production

High Purity Achieved with a Single Affinity Purification Step followed by Desalting

VHHs and minibinders were purified using a single-step IMAC affinity procedure followed by desalting in plate format. SDS-PAGE analysis demonstrated high purity for all constructs, with minimal background from components of the CFPS system (Figure 3). Quantitative analysis indeed indicated an average purity exceeding 80% (Graph 1). This high level of purity was sufficient for downstream biochemical and functional assays without the need for additional polishing steps.
The combination of high expression levels and efficient affinity purification underscores the robustness of the CFPS workflow. By eliminating multi-step purification strategies, the overall process is significantly accelerated while maintaining protein quality, making it particularly well suited for high-throughput screening and early-stage discovery workflows.

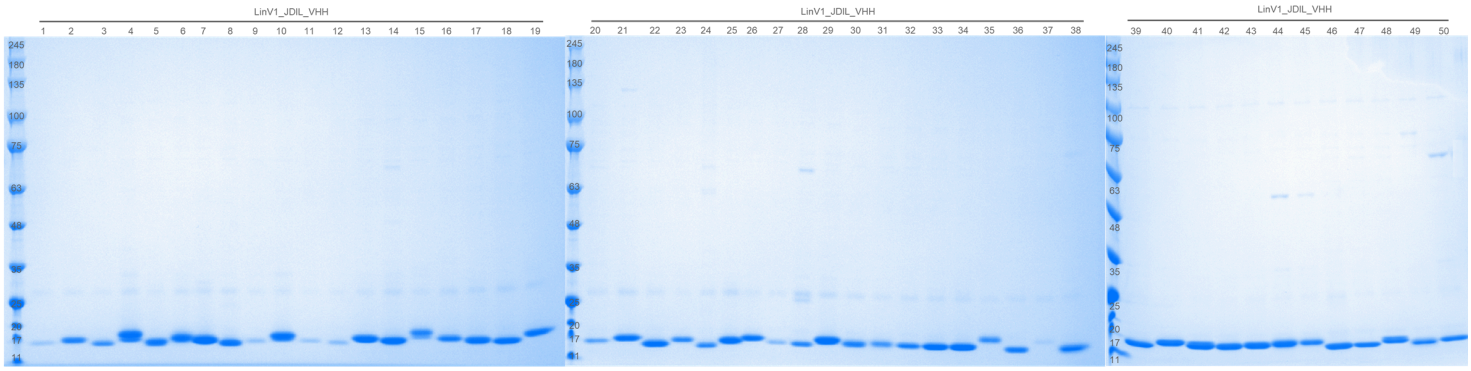


Figure 3 – SDS-PAGE analysis of the purified proteins. A total of 11.25 µL of each protein sample was loaded per well. The resulting band patterns confirm successful expression and a purity superior to 80% for each recombinant protein after purification.

CONCLUSIONS

The Syn-Xpress™ Gold VHH kit enables efficient, high-throughput expression of VHHs and minibinders in a cell-free system in less than 24 hours. The workflow described here — from codon-optimized linear DNA preparation, through CFPS reaction setup, to plate-based purification and rapid analytical assessment — provides a streamlined pipeline for early-stage screening and characterization.
Using plate-based IMAC purification followed by desalting, proteins of sufficient purity (>80%) and yield (0.1 to 0.6 µg/µL of CFPS) are obtained for downstream functional assays. The combination of A280 quantification and SDS-PAGE evaluation ensures rapid verification of expression levels, molecular integrity, and proper folding, supporting informed selection of lead candidates.
Overall, this approach reduces the time, labor, and resources required compared to traditional cell-based expression systems, while maintaining flexibility and scalability. It is particularly well suited for screening large VHH or minibinder libraries, accelerating discovery and enabling rapid progression from gene sequence to functional protein characterization.

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