# Case Study - SdAb Development for Soluble Protein Target

## Introduction

Single domain antibody (sdAb), is a kind of antibody fragments consisting of a single *monomeric* variable antibody domain and lacking the light chain and CH domain of the heavy chain in conventional Fab region. In terms of only 12-15 kDa molecular weight, which is much smaller than either full length antibody (150-160 kDa) or other antibody fragments (Fab ~50 kDa, scFv ~25 kDa), sdAb *takes* great advantages of stability and penetrability, which are essential to the development of several antibody drugs or diagnostic tools.

**Creative Biolabs** has been a long-term expert in the field of single domain antibody (sdAb) development. Our scientists have extensive experience in immunizing camelid animals with the target of interest to generate novel sdAbs. In terms of our advanced Hi-Affi™ phage display platform, we can use the immunized host animal to generate high-specific sdAbs for the interested targets. One animal immunized with one antigen is good enough to meet the majority of project requirements, which can offer a cost-effective option for specific sdAb development.

## Project Objective & Achievement

For this case study, one soluble protein (namely Target 1 or T1 for short) was provided as antigen and screening target. Creative Biolabs is entrusted to immunize one camel with Target 1 and then develop T1-specific single domain antibodies.

With the provided antigen, one camel was immunized. Promising immune response for Target 1 was observed after 5 injections, which is qualified for library construction. One uniform immune library was then constructed with the capacity of over $10^8$, a qualified level for library screening.

With the provided target, three rounds of biopanning were successfully performed with good enrichment. 40 clones were then randomly picked from the 3rd round enriched pool for validation. All the 40 clones were observed as positive through monoclonal phage ELISA and 37 unique $V_HH$ sequences have been identified. Except for clone 1 and 17, the other 35 clones were confirmed to specifically recognize Target 1 through and QC soluble ELISA.

Finally, there are 35 unique T1-specific sdAbs be discovered in this project.

## Milestone Overview

### Stage 1: Animal Immunization

One native (non-immunized before) camel was employed for this project. The immunization process was designed to last 91 days (5 injections with 3-week interval) and performed *via* multiple sites subcutaneous immunization strategy with increased antigen dosage, which contributes to triggering immune response for Target 1.

| Date | Steps | Date | Steps |
|---|---|---|---|
| Day 0 | Pre-bleed | Day 63 | 4th Injection |
| Day 0 | Primary Injection | Day 70 | Bleeding and Titration |
| Day 21 | 2nd Injection | Day 84 | 1st Boost Injection |
| Day 42 | 3rd Injection | Day 91 | Bleeding and Titration |
| Day 49 | Bleeding and Titration | Day 93 | Final Bleed |

**Table 1. Custom Designed Camel Immunization Schedule.**

After the 5th injection, test bleed was collected and 3rd titration was conducted to monitor the immune response. Target 1 was coated and tested in-parallel with pre-immune sera (negative control) and antisera. As shown in Figure 1, good immune response was observed for Target 1, the 3rd titer was over 1:25,600.

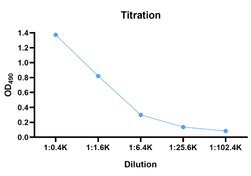


**Figure 1. 3rd titration results.**

### Stage 2: Library Construction

After 5th injection, the antisera were collected and subjected to PBMC isolation, RNA extraction, and cDNA preparation, freshly on the same day. The $V_HH$ genes were then PCR amplified by using our species-specific primers. The phagemid library was constructed with high-quality phagemid vectors and optimized ligation strategies to achieve 100% correct insertion rate. It was then desalted and subjected to electrotransformation with *E. coli* TG1 as the host strain to form the original bacteria library. 20 random clones were selected for QC colony PCR to identify the insertion of sdAb repertoire. Then 45 clones from the library were randomly picked and subjected to DNA sequencing and aligned, the results (omitted here) showed that no common sequences could be found among them. Based on the QC colony PCR and DNA sequencing analysis, a qualified immune library with the capacity of over $10^8$ has been generated successfully.

### Stage 3: Library Screening

Creative Biolabs can tailor a series of library screening strategies to find the best-fit one of your project. Our scientists are committed to collecting the most reliable data that contribute to understanding the actual situation of each step. For a typical screening process, pre-absorption will be performed before each round of screening to eliminate non-specific binders against the plate surface, corresponding blocking buffer, and negative target (if exists) as much as possible. From the second round, "No Coating" control is also performed in parallel with the "Target Coating" group. If there is any negative target required by the project, an in-parallel test of "Negative" control will be involved as well from the second round.

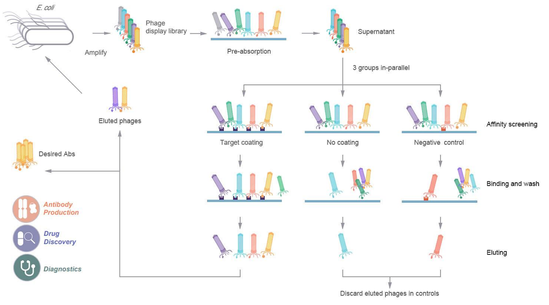


**Figure 2. Flow diagram of phage display-based screening.**

For this case study, solid-phase screening strategy was performed, which the targets were immobilized on the plate surface directly and screened separately. After three rounds of biopanning, good enrichment was observed for Target 1 and clear difference was found between the "Target Coating" group and "No Coating" control (Figure 3). This indicated some specific binders have been selected for Target 1.

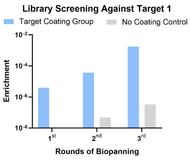


**Figure 3. Process monitoring of library screening stage.**
(Enrichment is increased round by round and presents significant difference with no coating control.)

### Stage 4: Binder Validation

After the biopanning, 40 clones were randomly picked from the 3rd round output of the target group. The monoclonal phage ELISA was then performed against the target.

For Target 1, 40 positive clones were observed and then processed for DNA sequencing (Figure 4). 37 unique clones were identified (Figure 5). All these unique clones were then prepared as soluble format (phage-free) for the validation of QC soluble ELISA. As shown in Figure 6, 35 clones were finally confirmed to recognize the target positively except clone 1 and 17.

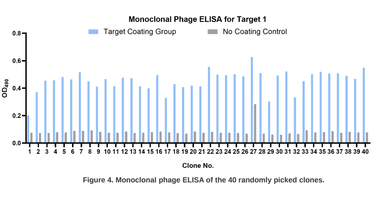


**Figure 4. Monoclonal phage ELISA of the 40 randomly picked clones.**

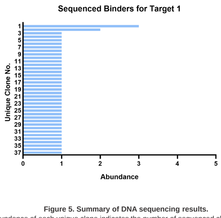


**Figure 5. Summary of DNA sequencing results.**
(Abundance of each unique clone indicates the number of sequenced clones present the same sequencing information.)

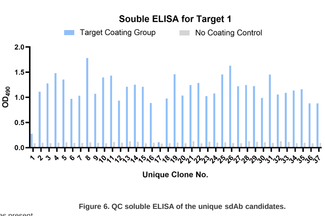


**Figure 6. QC soluble ELISA of the unique sdAb candidates.**

## Conclusion & Key Words

- ✓ **Soluble Protein Target** - Soluble protein antigens can be immunized for phage display library generation and novel sdAb discovery.
- ✓ **High-Quality SdAb Library** - Creative Biolabs' Hi-Affi™ platform can contribute to generating immune library with maximized diversity and capacity.
- ✓ **High Fidelity Screening** - Solid-phase strategy combined with in-parallel control groups, which achieved great enrichment and support the reliability of the screening outcomes.
- ✓ **Two-Step Validation** - Antigen-specific clones were identified and validated through both monoclonal and soluble ELISA, which can avoid potential false positive.
- ✓ **One-Stop Solution** - Extensive experience and integrated procedure enable our scientists to smoothly advance the project and meet all your objectives.

## Contact Us

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