Case Study - Anti-ID antibody development

Introduction

Anti-ID Abs are Abs that can recognize the variable region of an Ab and produce specific binding. Anti-ID Abs play a vital role in the development of drugs. On the one hand, anti-ID Abs can serve as a valuable reference for immunogenicity analysis. On the other hand, they are also vital tools for pharmacokinetic/pharmacodynamics (PK/PD) studies. Creative Biolabs offers unparalleled anti-ID Abs production and downstream testing services. We are committed to developing anti-ID Abs with high specificity, high affinity and high sensitivity from different species, such as rabbit, chicken, cow, sheep, mice, rats and humans to meet your unique needs.

Idiotype:
The specific combination of idiotopes present within an antibody's complement determining regions (CDRs). A single idiotope, is a specific region within an antibody's Fv region which binds to the paratope (antigenic epitope binding site) of a different antibody.

Anti-idiotype antibody:
An anti-idiotypic (Anti-ID) antibody binds to the idiotype of another antibody, usually an antibody drug.

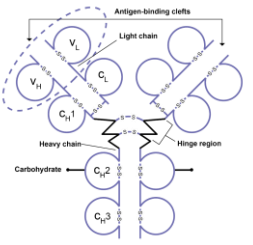

Fig.1 Ab Structure Analysis.

Project Objective & Achievement

The client provided the full-length antibody. Creative Biolabs was contracted to prepare Fab antigen and generate anti-idiotypic antibodies through mice immunization and hybridoma screening.

The project is divided into the following stages, namely Fab digestion, mouse immunization, then cell fusion, hybridoma screening and subcloning, competitive activity detection, expansion of the suitable hybridoma candidates, and antibody production.

Five mice were employed for immunization, and each mice received 50 μg antigen. After the 4th injection, blood samples were collected and the titer of antiserum against the target and isotype control antibody was determined by indirect ELISA. Mouse 2 that presents the best titer was selected for the cell fusion.

A total of ~1,1000 clones were identified and tested by ELISA (11 96-well cell culture plates ~). After the 1st round specific-ELISA screening, 205 clones showed strong positive signal towards the target Fab and no binding signal against isotype control antibody. After 2nd round specific-ELISA screening, 77 strains were finally selected.

Then, we performed hybridoma subclone twice. Finally, 21 positive strains were identified. After competitive activity detection, we obtained 10 positive clones with competitive activity and 10 clones with non-competitive activity against target protein.

Finally, ascites antibodies were produced with the clones our customer selected.

Project Overview

Stage 1: Fab Preparation

For Fab preparation, Creative Biolabs digested the full-length IgG antibody and purified the Fab. The purity of purified Fab is over 90%.

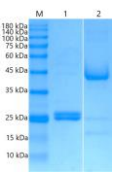

Fig.2 SDS analysis of Purified Fab
M: Marker
Lane 1: Fab-reducing conditions
Lane 2: Fab non-reducing conditions

Stage 2: Animal Immunization

Five mice were employed for this project. The immunization process was planned to last 65 days (5 injections with 2-week interval) and performed *via* multiple sites subcutaneous immunization strategy. Among the whole process, ELISA titration was performed after 3rd and 4th injection respectively.

Table 1. Typical Immunization Schedule.

Date	Steps	Date	Steps
Day 0	Pre-bleed	Day 45	4th Injection
Day 0	Primary Injection	Day 52	Bleeding and Titration
Day 15	2nd Injection	Day 60	5th Injection
Day 30	3rd Injection	Day 67	Bleeding and Titration
Day 37	Bleeding and Titration		

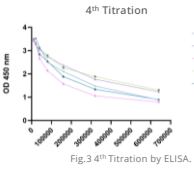

Fig.3 4th Titration by ELISA.

Stage 3: Competitive Activity Detection by ELISA

For competitive activity detection, Creative Biolabs performed the Competitive ELISA assay by coating the target Ab on the plate, then adding target protein with serial dilution or PBS to detect the competitive activity.

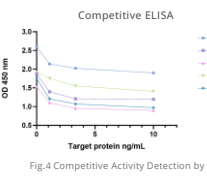

Fig.4 Competitive Activity Detection by ELISA.

Stage 4: Hybridoma Screening

Mice which presented the best titer was chosen for the cell fusion. Creative Biolabs can tailor a series of 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 hybridoma screening, indirect ELISA was firstly performed before any customized screening.

Positive clones obtained in the first round of screening will be subjected to a second target specific screening. After second round of ELISA screening, selected positive hybridomas will be subcloned and tested again. The ELISA result of the second round specific ELISA screening was shown in Figure 5.

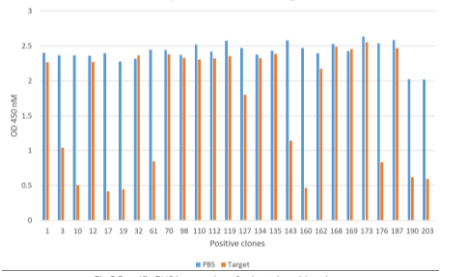

Fig.5 Specific ELISA screening of selected positive clones.

Stage 4: Hybridoma Subclone

After two rounds hybridoma screening, Creative Biolabs performed hybridoma subclone twice. The supernatant antibody was tested against desired target and isotype control antibody. The ELISA result of the finally selected hybridomas are shown in the Figure 6.

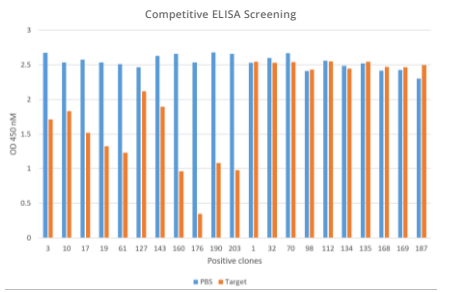

Fig.6 Specific ELISA screening of selected positive clones.

Stage 5: Function Test of Selected Positive Clones

The culture supernatants of all positive hybridomas will be delivered for customized assay tests. After verifying the function of the delivered supernatant, Creative Biolabs can provide ascites antibody preparation services, hybridoma sequencing services and recombinant antibody expression services for the designated clones.

Antibodies have become essential tools for research use due to their high specificity, high binding affinity, long half-lives, and low toxicity. High-quality anti-ID antibody development services at **Creative Biolabs** have covered you for your pharmacokinetics, anti-drug-antibody assay, immunogenicity evaluation and other assay needs. A personalized and flexible solution will be designed and adapted to your needs.

Stage 6: *In Vivo* Antibody Production & Affinity Measurement

After supernatant validation by client, two clones were selected for further antibody production and purification. The antibody purity was determined by SDS-PAGE respectively and the antibody integrity was good. Antibody affinity measurement result was shown in Figure 6.

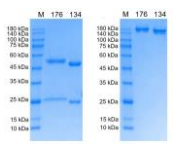

Figure 7. SDS analysis of purified Antibodies.

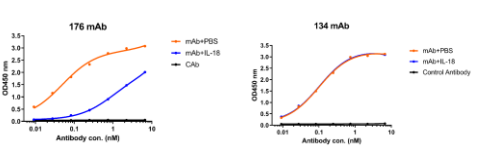

Figure 8. Competitive Activity Detection by ELISA.

Table 2. Purified Antibody Summary.

Item	Description	Concentration	Quality	EC50	
				mAb+PBS	mAb+Target
1	Clone 176 mAb	2.6 mg/mL	5 mg	0.0490 nM	0.9164 nM
2	Clone 134 mAb	2.6 mg/mL	5 mg	0.1007 nM	0.1033 nM

Highlights

- **One-Stop Solution** - Extensive experience and integrated procedure enable our scientists to smoothly advance the project and meet all your objectives.
- **High-Quality Screening Services**- different types anti-ID antibodies screening in one program, >90% success rate
- **Personalized customized services**-Monoclonal or Polyclonal; IgG or F(ab)$_2$; Competitive ELISA Assay methods, Competitive or non-Competitive antibodies

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