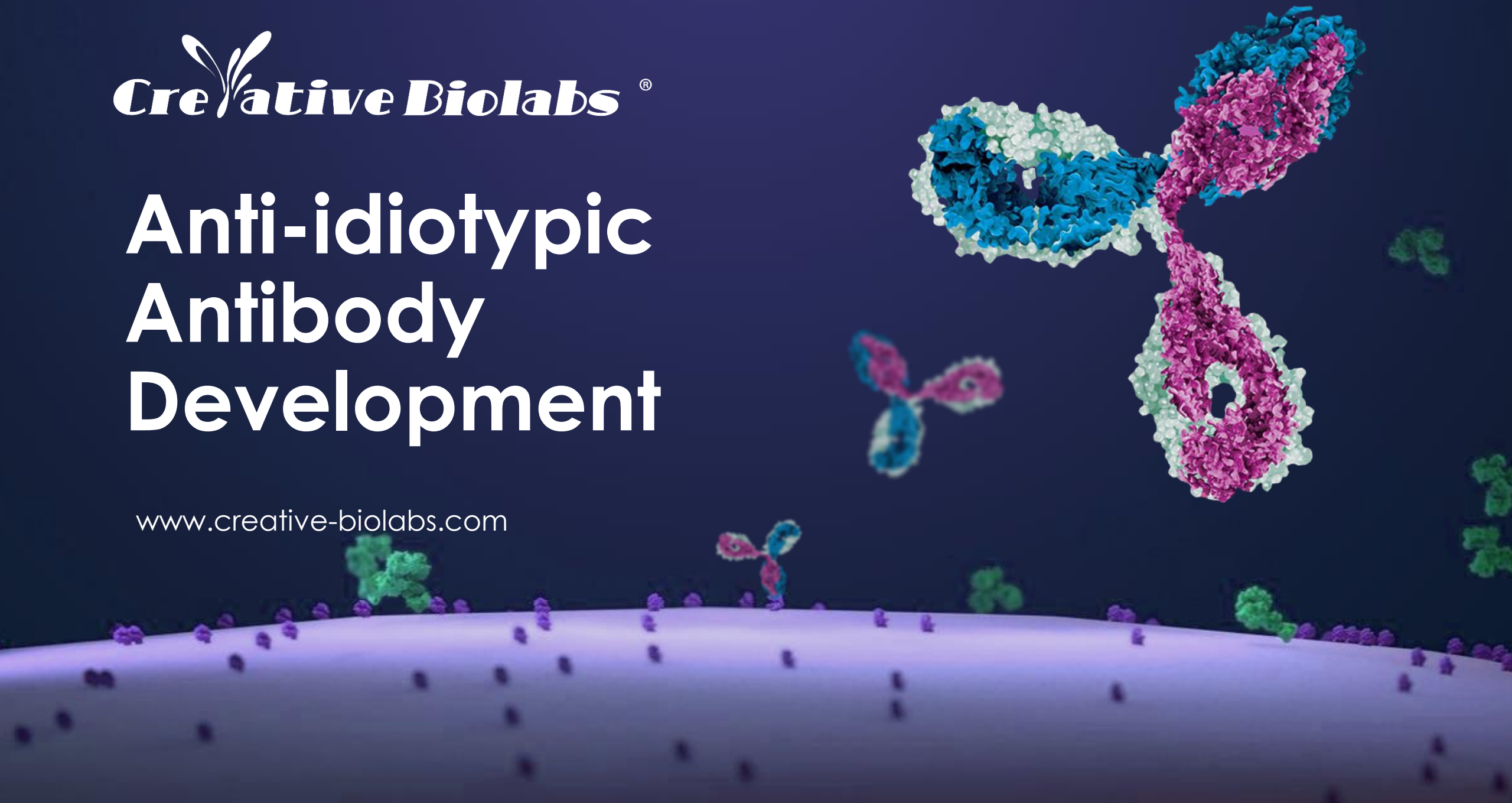
Creative Biolabs®

# Anti-idiotypic Antibody Development

www.creative-biolabs.com

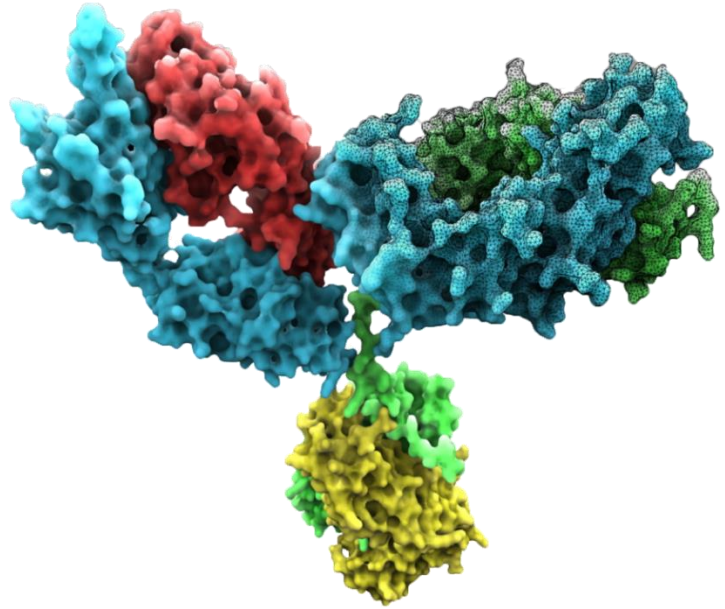


# About Us

*"Creative Biolabs is committed to providing highly customized comprehensive solutions with the best quality to advance our global clients' projects."*

Creative Biolabs is the leading custom service provider that has extensive experience in various antibody production and engineering fields. Our service portfolio includes mouse and rat monoclonal antibody production using hybridoma technology, human, monkey, rabbit, chicken, dog, llama and camel monoclonal antibody production using various antibody library technologies (including phage display, bacterial display and yeast display). We are also professional in conducting in depth antibody humanization and affinity maturation using phage display and DNA mutagenesis approaches.

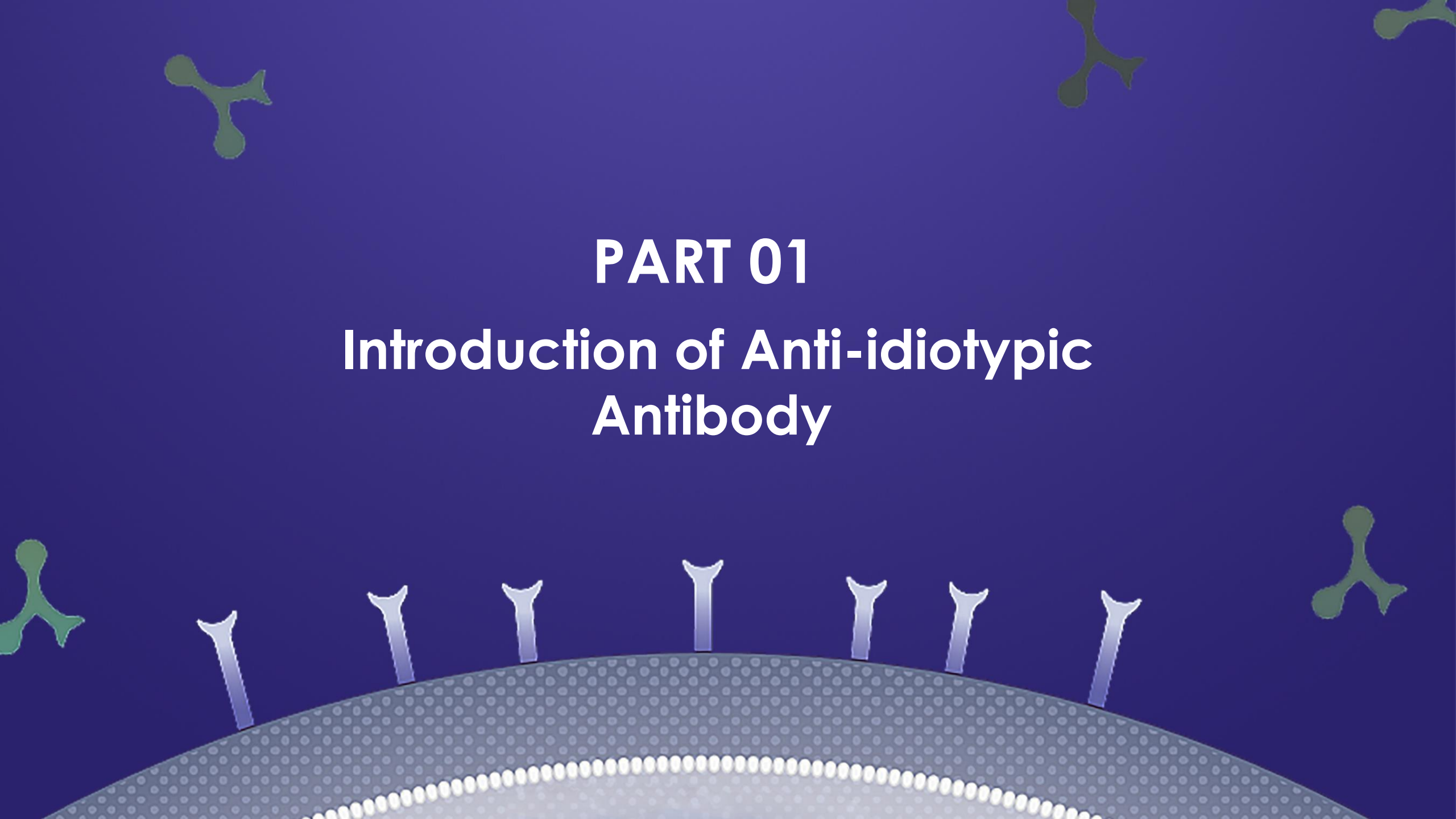
We also have rich experiences in anti-Idiotypic antibody production. With our well-established antibody development platform, we can customize anti-ID antibodies according to your project requirements, such as PK assay and ADA assay.



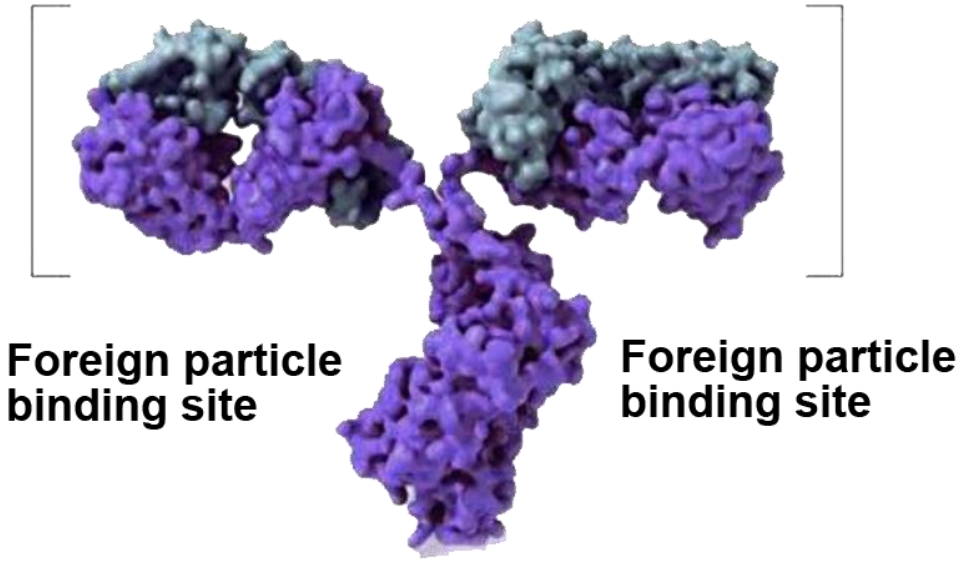
# Contents

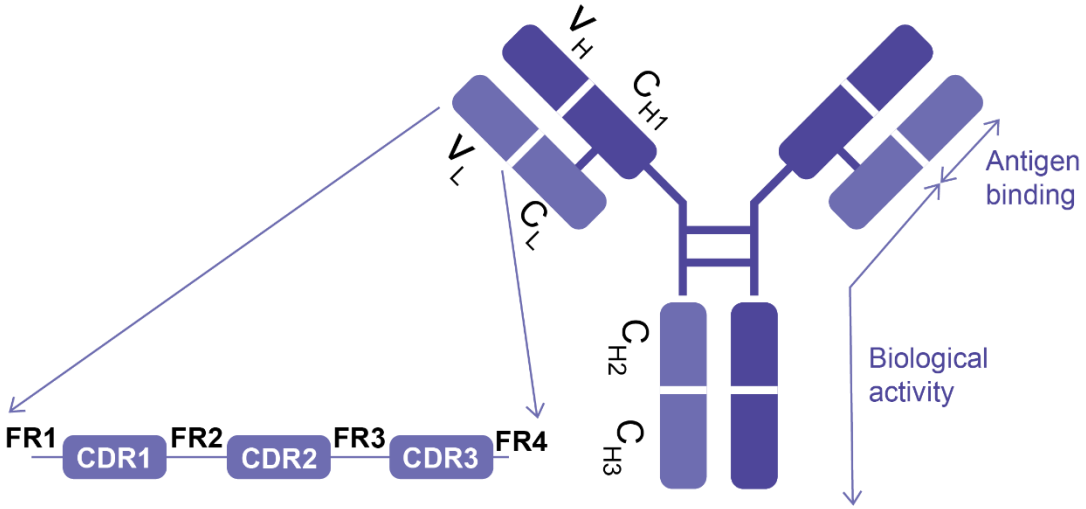
# PART 01

# Introduction of Anti-idiotypic Antibody

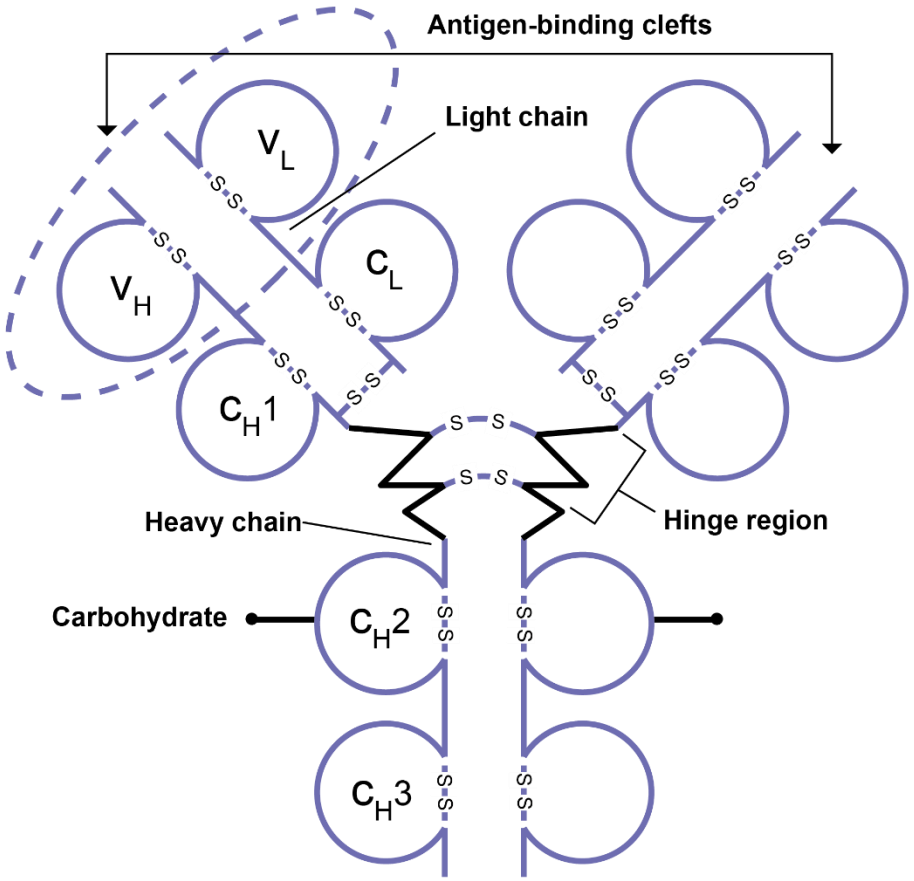


# Antibody Structure Analysis



# Anti-idiotypic Antibody



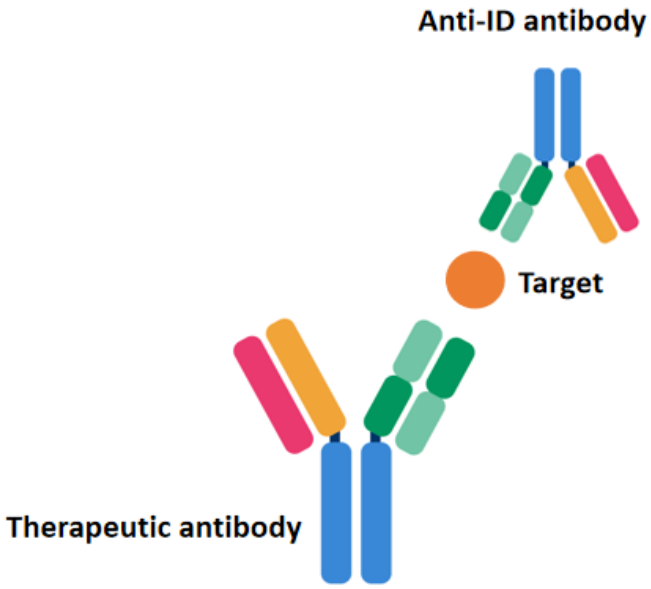

## Idiotype:

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:

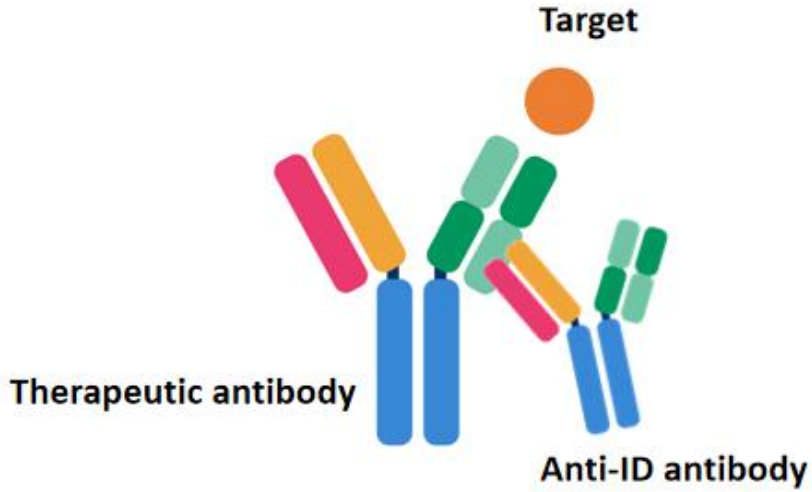
an anti-idiotypic (Anti-ID) antibody binds to the idiotype of another antibody, usually an antibody drug.

# Types of Anti-idiotypic Antibody




Type1. Anti-ID Antibody Detects **Free Antibody**

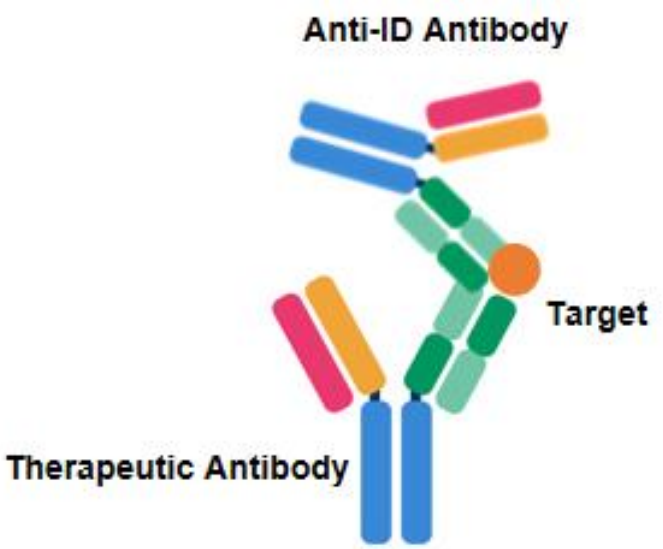
- Paratope specific
- Inhibit antigen binding
- Neutralizing original antibodies
- Antigen mimicry effects




Type 2. Anti-ID Antibody Detects **Total Antibody**

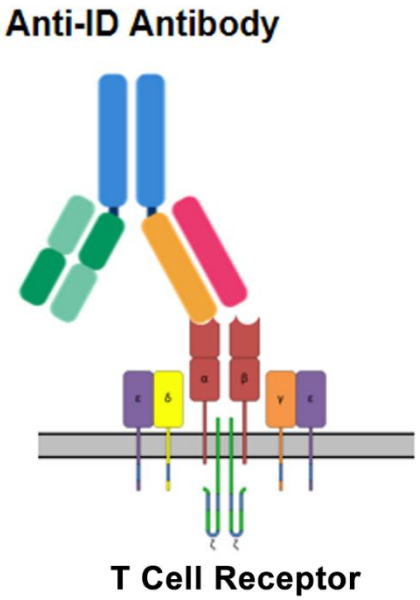
- Non-paratopic epitope
- Detect free, partially bound, fully bound antibodies
- Non-inhibitory to antigen binding

# Types of Anti-idiotypic Antibody




Type 3. Anti-ID Antibody Detects **Bound Antibody Exclusively**

- Partially paratopic specific/antibody-antigen complex specific
- Detect partially bound, fully bound antibodies
- Non-inhibitory to antigen binding

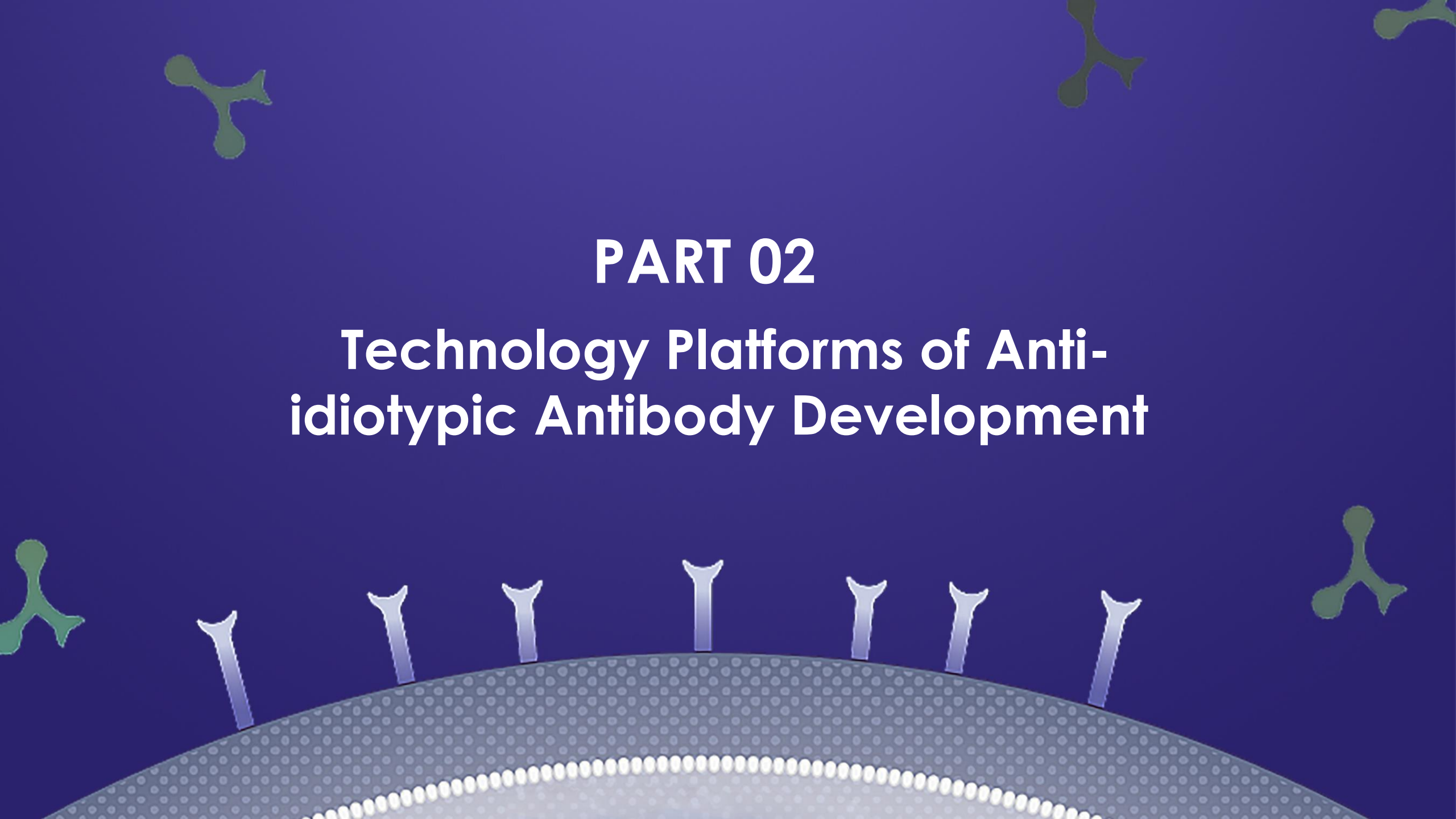



Type 4: Anti-ID Antibody Detects **T Cell Receptor**

- TCR extracellular domain specific
- Immune modulation potential

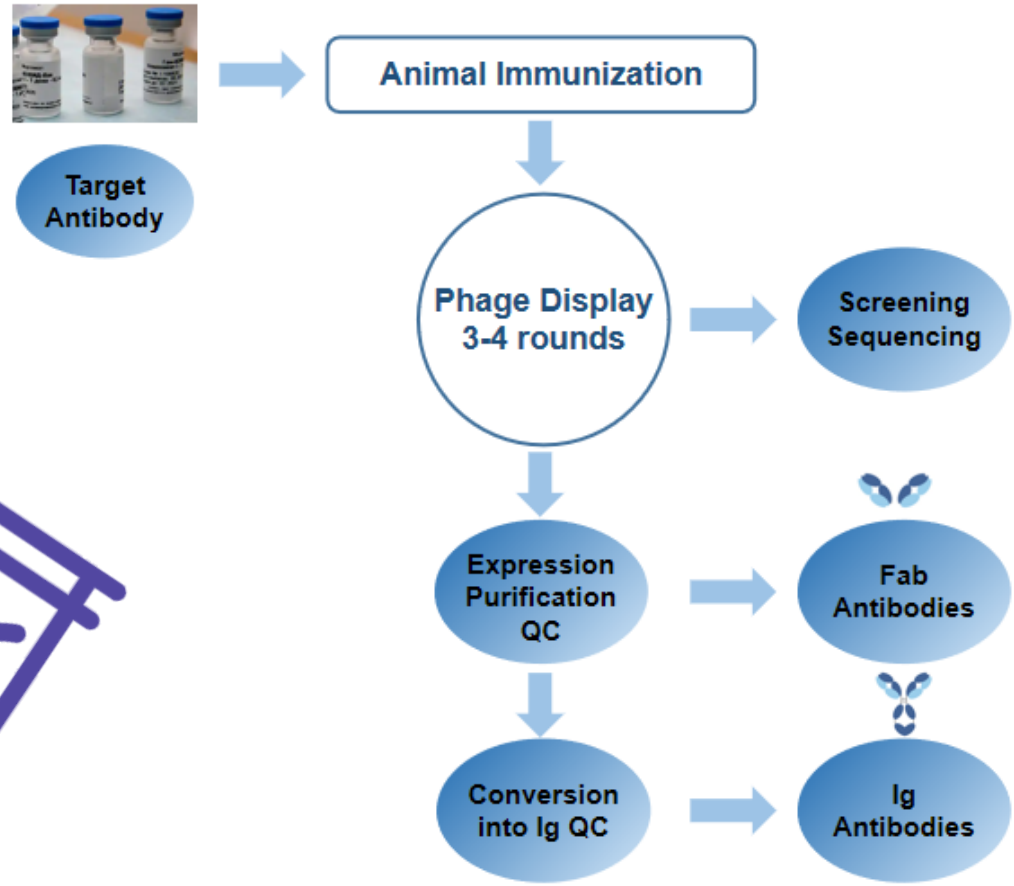
# PART 02

# Technology Platforms of Anti-idiotypic Antibody Development



# Immunized Antibody Library Technology

This is the best approach that can raise a large number of high-affinity anti-ID antibodies. Target antibody in the forms of whole IgG, scFv, Fab, F(ab')2 , VHH or peptides designed according to the sequences of the variable domains is utilized to immunize animals.




## Features:

- Diverse antibody repertoires
- Antigen-specific libraries
- Affinity matured
- Counter selections available
- **Guided selection** to get different types of anti-ID antibodies

# Native Antibody Discovery

Creative Biolabs has developed the unique Native® Antibody Discovery Platform to discover native monoclonal antibodies using antigen-specific B lymphocyte cytometry technology plus antibody gene cloning from single cells. Both plasma and memory B cell sorting methods are employed.

## Features:

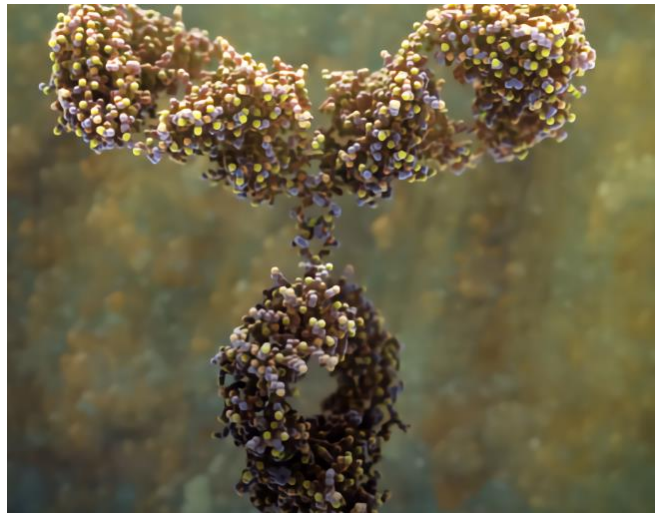
- Native paired heavy chain and light chain
- High affinity due to *in vivo* affinity maturation
- High selectivity and stability *in vivo*
- Large pool of antibody candidates

# Premade Phage Displayed Library Screening

Screening our premade phage display antibody libraries can be a time-saving approach to generating anti-idiotype antibodies that recognize the target antibodies in native conformation.

If isotype matching control antibodies are available, we can use them to deplete/block the binders that target the constant regions of the target antibody.

- **Naive libraries**: constructed from non-immunized host (human, mouse, rat, rabbit, llama, camel, shark, etc.)
- **Synthetic libraries**: constructed from antibody framework with randomized CDR regions.



# Premade Phage Displayed Library Screening

## Features

- **Plentiful library resources** with large capacity and diversity, which greatly facilitates the discovery of ideal binders
- Particularly suitable for human native antigens, **antigens that are difficult to immunize**, or antigens that cannot elicit potent immune response in other animals
- **Highly efficient and robust**
- **Various screening targets**: proteins/peptides, small molecules, living whole cells, membrane protein reconstitution formats, etc.
- **Guided selection** to get different types of anti-ID antibodies

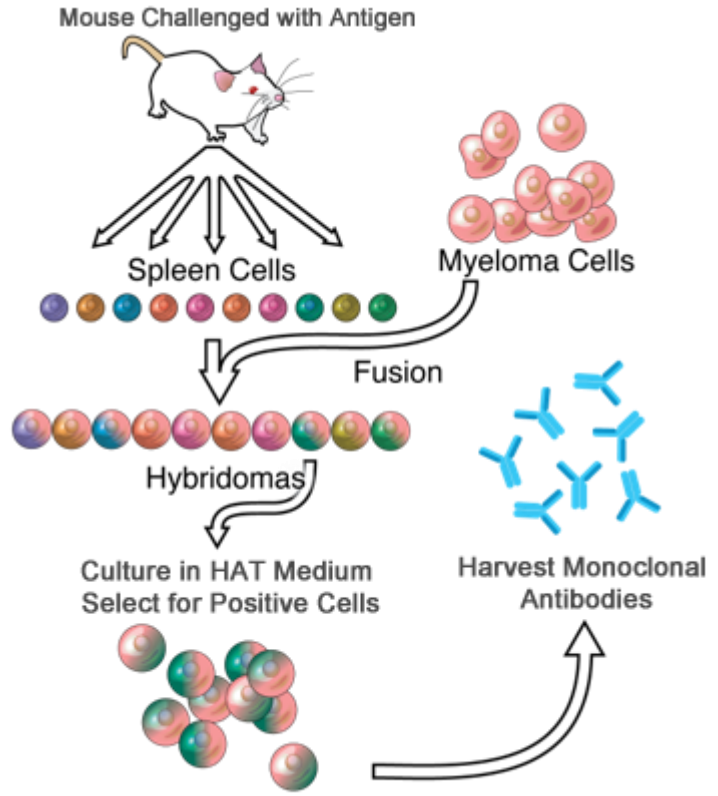
# Premade Phage Displayed Library Screening

| Libraries | Display Technology | Library Format | Specise | Library Size |
|---|---|---|---|---|
| HuScL-2 | Phage Display | Semi-synthetic scFv | Human | $1.42\times10^{9}$ |
| HuScL-6 | Phage Display | Naïve scFv | Human | $1.0\times10^{11}$ |
| HuScL-7 | Phage Display | Naïve scFv | Human | $1.1\times10^{10}$ |
| HuScL-2S | Phage Display | Semi-synthetic scFv | Human | $2.36\times10^{10}$ |
| HuFabL-4 | Phage Display | Naïve Fab | Human | $1.9\times10^{10}$ |
| HuFabL-5 | Phage Display | Naïve Fab | Human | $1.1\times10^{10}$ |
| HuFabssL-1 | Phage Display | Naïve & synthetic Fab | Human | $1.8\times10^{10}$ |
| MuScL-1 | Phage Display | Naïve scFv | Mouse | $8.0\times10^{8}$ |
| MuScL-2 | Phage Display | Naïve scFv | Mouse | $5.3\times10^{10}$ |
| MuFabL-1 | Phage Display | Naïve Fab | Mouse | $6.0\times10^{9}$ |
| RaFabL-1 | Phage Display | Naïve Fab | Rabbit | $7.5\times10^{9}$ |
| RaFabL-2 | Phage Display | Naïve Fab | Rabbit | $1.2\times10^{10}$ |
| Chicken-ScL-1 | Phage Display | Naïve scFv | Chicken | $1.2\times10^{9}$ |
| CaVHHL-1 | Phage Display | Naïve VHH | Camel | $1.5\times10^{9}$ |
| CaVHHL-3 | Phage Display | Naïve VHH | Camel | $3.0\times10^{9}$ |
| CaVHHL-4 | Phage Display | Naïve VHH | Camel | $2.63\times10^{10}$ |
| LlaVHHL-1 | Phage Display | Naïve VHH | Llama | $2.0\times10^{9}$ |
| LlaVHHSS-1 | Phage Display | Synthetic VHH | Llama | $1.0\times10^{11}$ |

# Hybridoma

To raise hybridoma clones in rats or mice using the target antibody as the immunogen, and to subsequently use isotype matching control antibodies to do counter-selection is another classic strategy to produce high specific, high affinity anti-idiotypic antibodies. We usually cut the target antibody into Fab to be used as immunogen. Here, a typical mistake is to take the whole antibody to raise hybridomas; this way, the Fc part of the whole antibody will dominate the immune response.

Currently we can provide first-class custom antibody & hybridoma service using a variety of species:

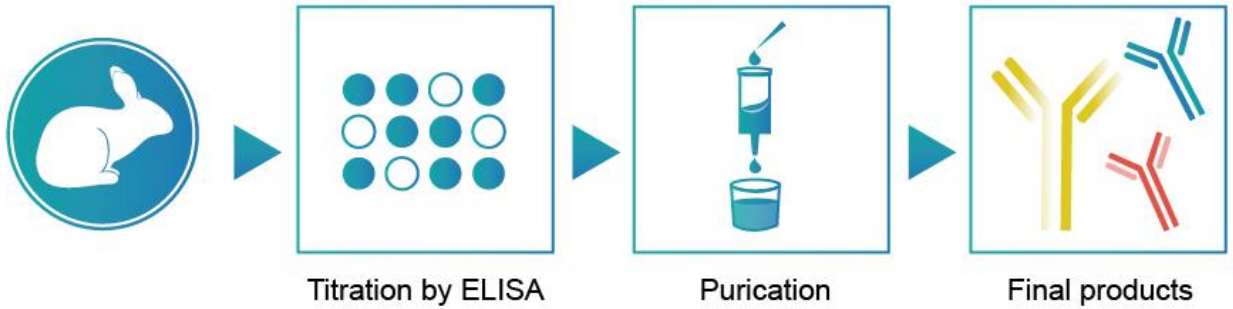



# Rabbit Polyclonal Antibody Platform

Creative Biolabs offers rabbit anti-idiotypic antibody production service. We have established a world-leading platform for rabbit anti-idiotypic antibody development. Rabbit anti-ID polyclonal antibody is usually used to detect total antibodies and it can be produced in a short time.

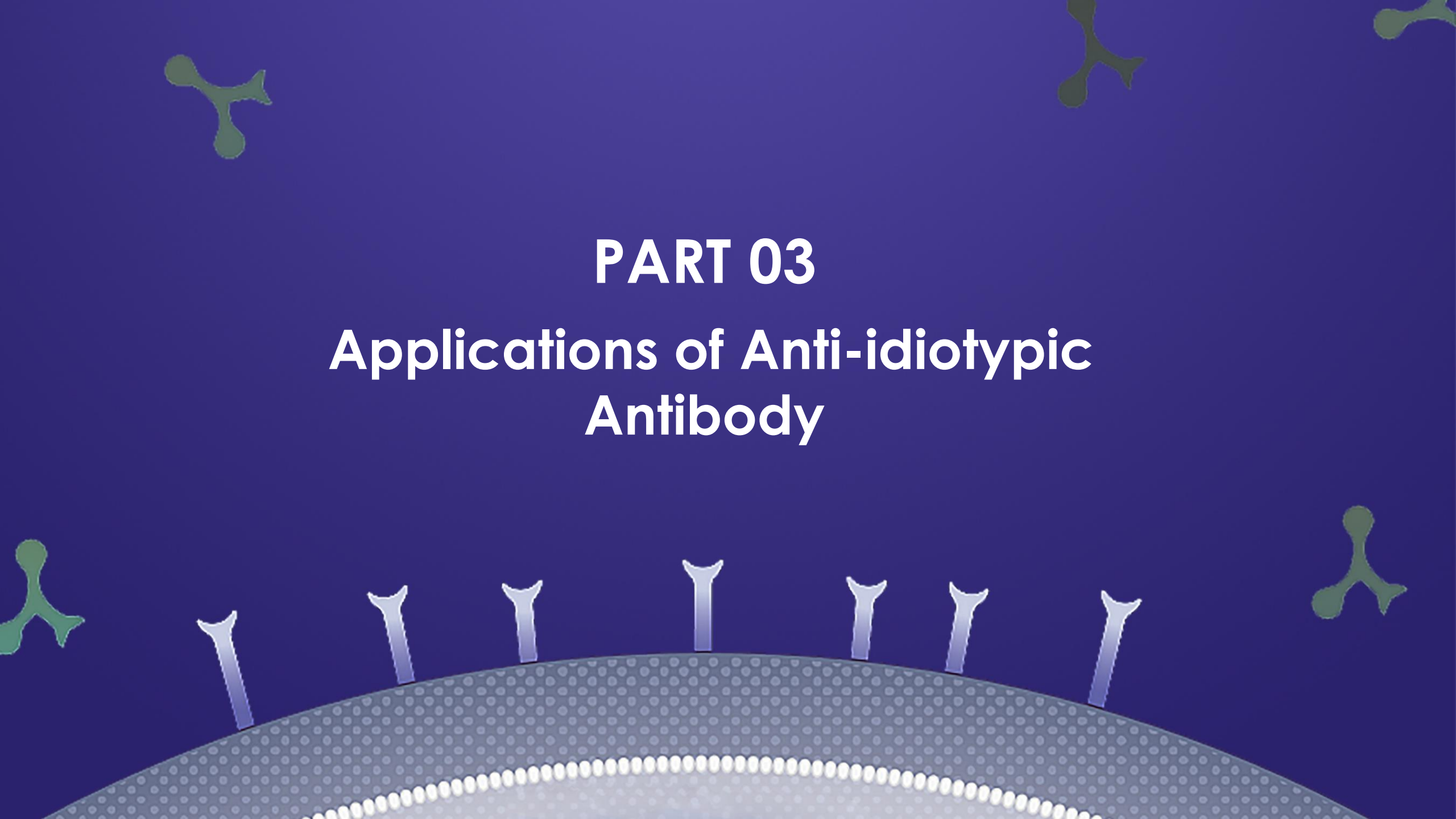
Advantages of rabbit anti-idiotypic antibody:

- Can simulate real conditions in blood samples
- The preparation cycle is relatively short
- Low cost



# PART 03

# Applications of Anti-idiotypic Antibody

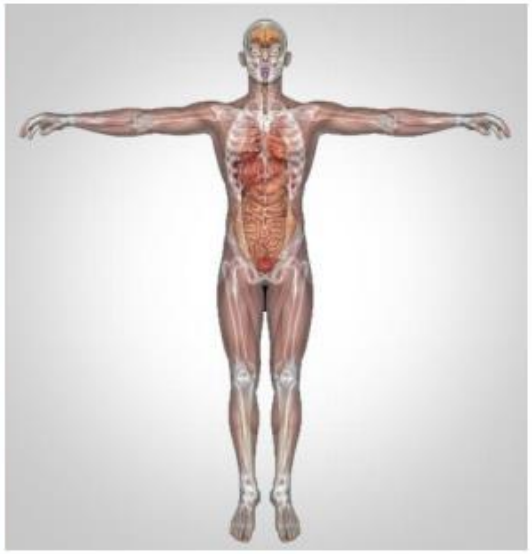


# Antibody Drug Development

**Pharmacokinetics, PK**
Detection reagent

**Anti-drug-antibody assay, ADA**
Positive control or test standard

# Pharmacokinetic (PK) Assay



Detection of plasma concentrations in animal or human serum

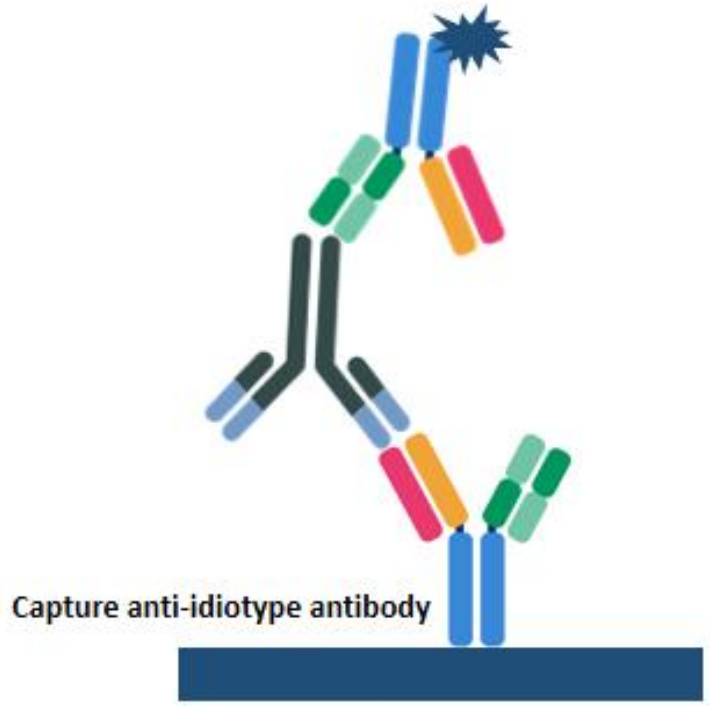
Analyze the absorption, distribution, metabolism and elimination of drugs in animals or humans

- Preclinical initial dose setting

- Analysis of drug interactions and concentration monitoring
- Clinical safety and efficacy evaluation

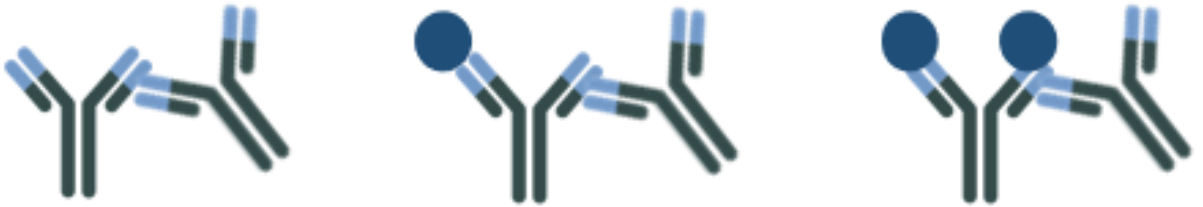
- Drug dosage improvement
- Drug use guidence

# Drug Concentration Detection




Anti-idiotype capture ELISA

**Competitive anti-ID antibodies**-Detection of free antibody drugs and partially bound antibody drugs



**Non-competitive anti-ID antibodies-**Detection of total antibody drugs

# Immunogenicity Evaluation

## Binding

- Bind with drugs
- Interfere with PK and TK drug testings
- Cause hypersensitivity reactions

## Sustaining/Clearing

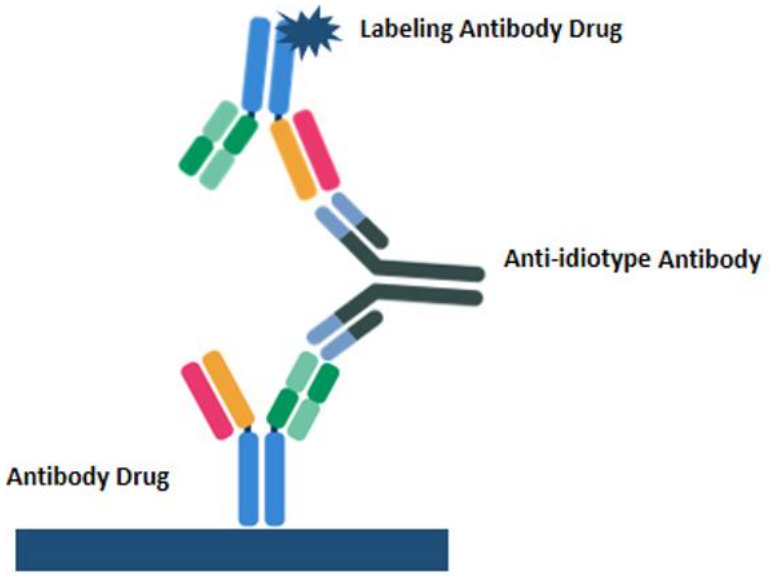
- Form complexes with drugs, prolong/shorten drug half-life
- Extend/reduce drug exposure time

## Neutralizing

- Form complexes with drugs, preventing drugs target-target binding
- Decreased efficacy

## Common detection methods for immunogenicity evaluation:

- Bridging ELISA
- Radioimmunoprecipitation (RIPA)
- Surface Plasmon Resonance (SPR)
- Electrochemiluminescence Detection (ECL)

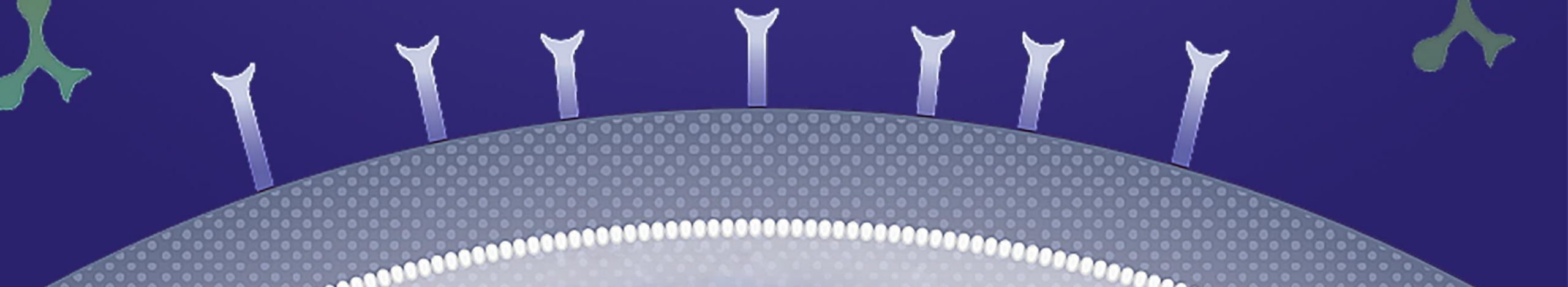



## Principles for establishing immunogenicity testing methods:

- High sensitivity (clinical 100 ng/mL, preclinical 250-500 ng/mL)
- Can cover all subtypes of ADA antibody detection (IgG, IgM, IgE, etc.)
- Anti-ID antibody is used as positive control antibody to establish detection methodology validation
- Set up negative individual controls (unmedicated individuals, healthy individuals/target disease groups)

# PART 04

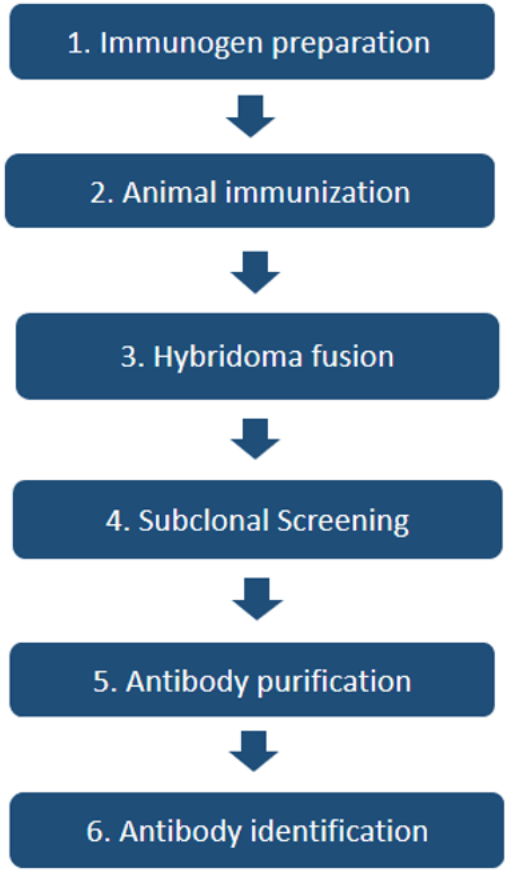
# Anti-idiotype Antibody Development and Difficulty Analysis



# Choice of Anti-ID Antibody Type: Monoclonal or Polyclonal Antibodies?

| Item | Anti-ID Monoclonal Antibody | Anti-ID Polyclonal Antibody |
|---|---|---|
| Application | PK detection | Immunogenicity evaluation |
| Timeline | 12~29 weeks | 11~17 weeks |
| Advantages | • Single epitope<br>• High specificity<br>• High batch-to-batch stability | • Can simulate real conditions in blood samples<br>• Relatively short preparation cycle<br>• Low cost |
| Disadvantages | • Long preparation cycle<br>• Relatively high cost<br>• Does not reflect the true condition of blood samples | • Low specificity<br>• Low batch-to-batch stability |

# Anti-ID Monoclonal Antibody Development Process and Difficulty Analysis




Main difficulties:

- How to choose an immunogen;
- How to improve the immune response of the CDR region;
- How to improve the success rate of anti-ID antibody fusion;
- Anti-ID antibody detection method;
- How to distinguish different types of anti-ID antibodies in the early stage of screening;
- Protocol for the identification of purified antibodies.

# Anti-ID Monoclonal Antibody Development Process and Difficulty Analysis

## 1. Immunogen selection - IgG, $F(ab)_2$

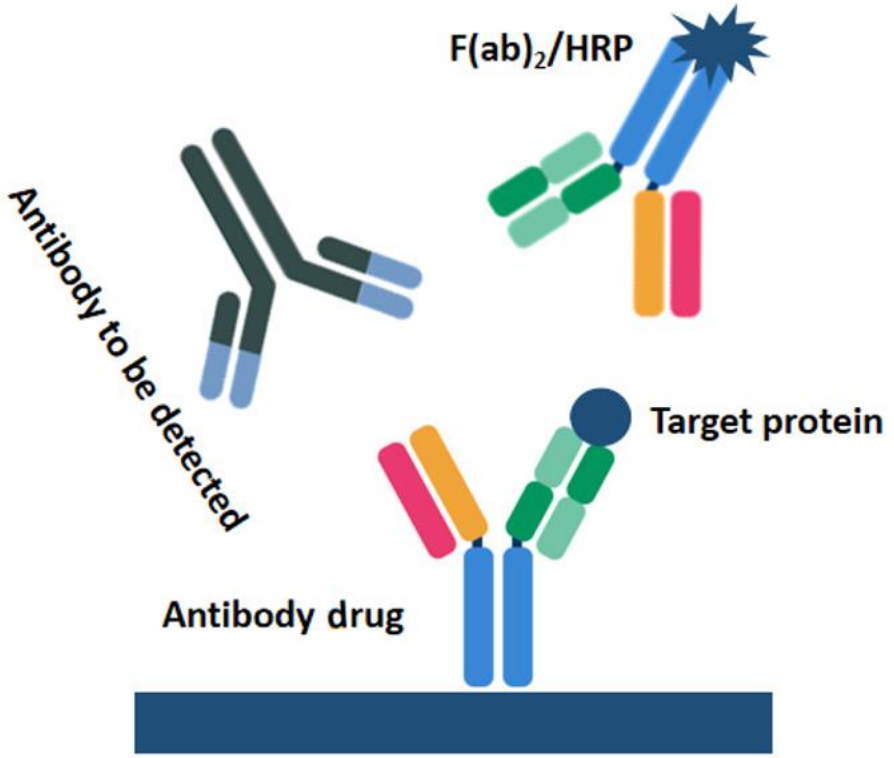
| Immunogen | IgG | $F(ab)_2$ |
|---|---|---|
| Cross-react with isotype IgG | ++ | + |
| Cost | + | ++ |
| Recognize antibody drugs | ++ | + |
| Probability of obtaining both types of antibodies | + | ++ |

## 2. Improve immune response for CDR region

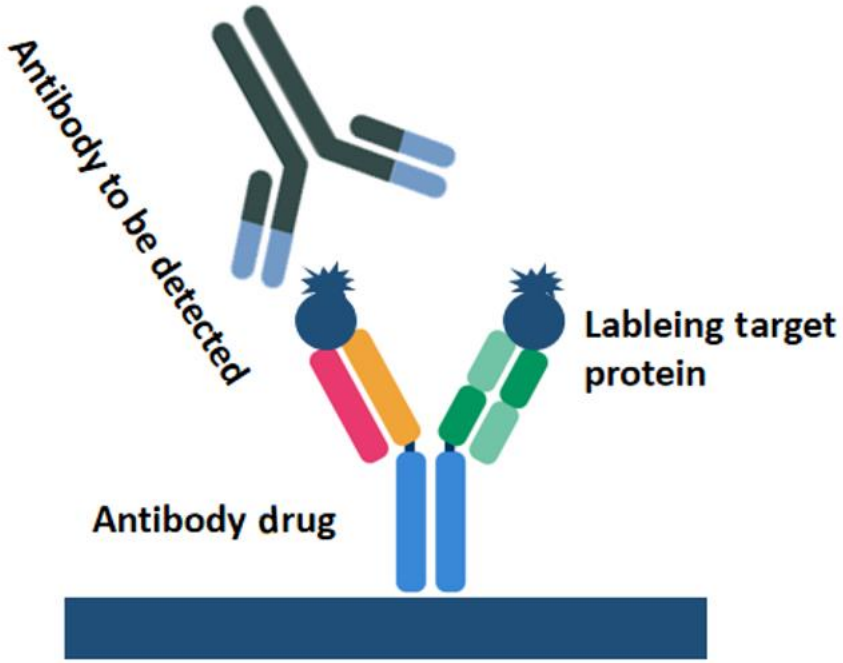
- Use the digested $F(ab)_2$ for immunization—reduce the proportion of non-specific antibodies caused by the Fc fragment;
- Try a variety of immune adjuvants—Freund's adjuvant, aluminum adjuvant, QS21, titermax, etc.;
- Adjust the immune cycle;
- Try macromolecular protein conjugation post-immunization (KLH, etc.)

# Anti-ID Monoclonal Antibody Development Process and Difficulty Analysis

## 3. Anti-ID antibody typing detection method

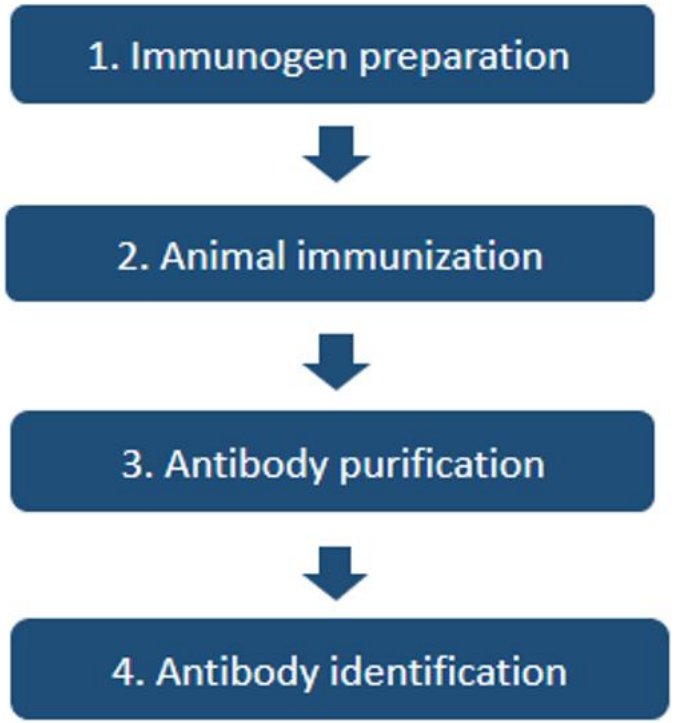



Competitive ELISA Assay-1




Competitive ELISA Assay-2

# Anti-ID Polyclonal Antibody Development Process and Difficulty Analysis

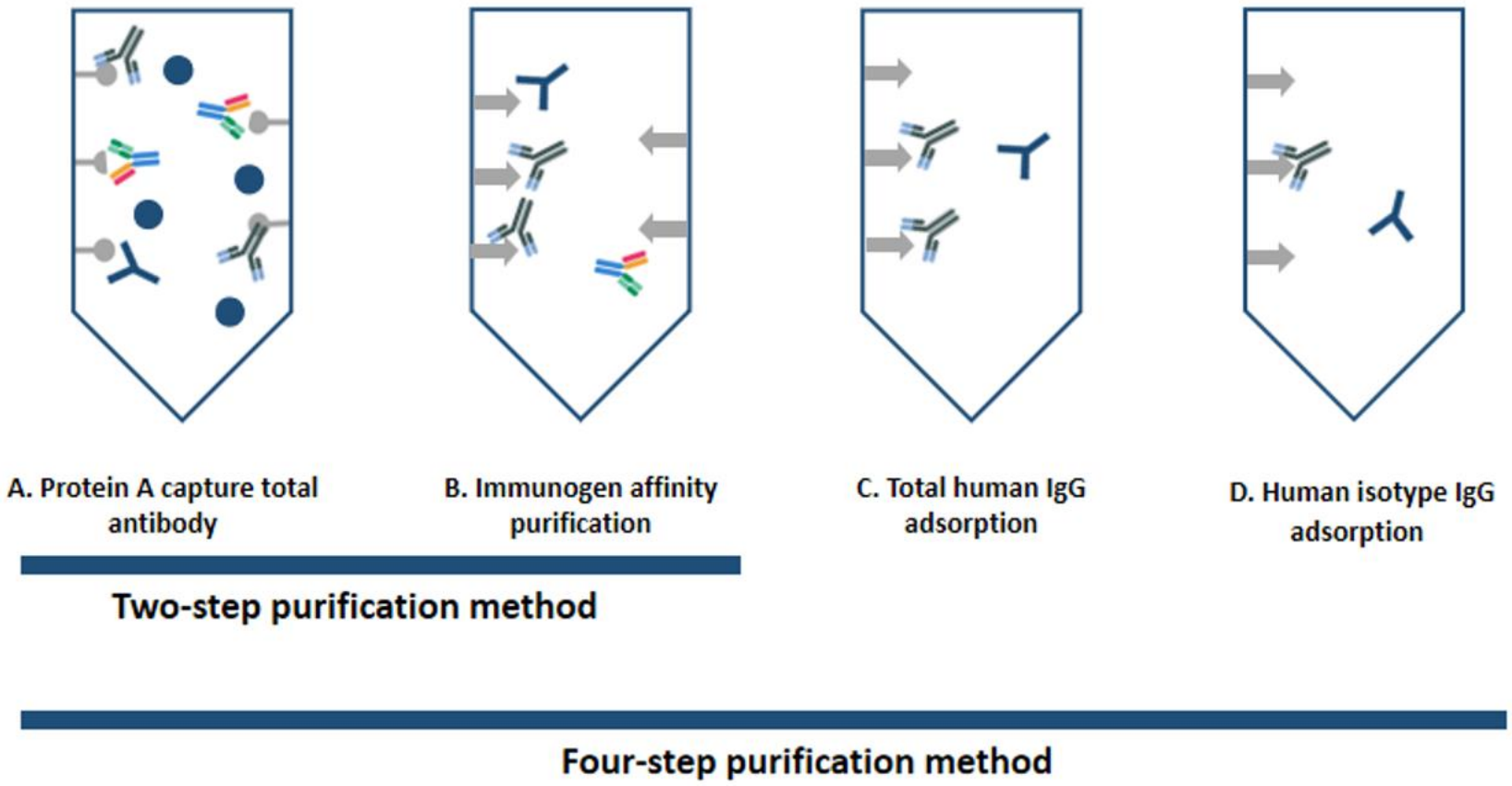



Main difficulties:

- How to choose the immunogen of polyclonal antibody;
- Anti-ID polyclonal antibody purification scheme;
- How to choose the purification method according to the intended application;

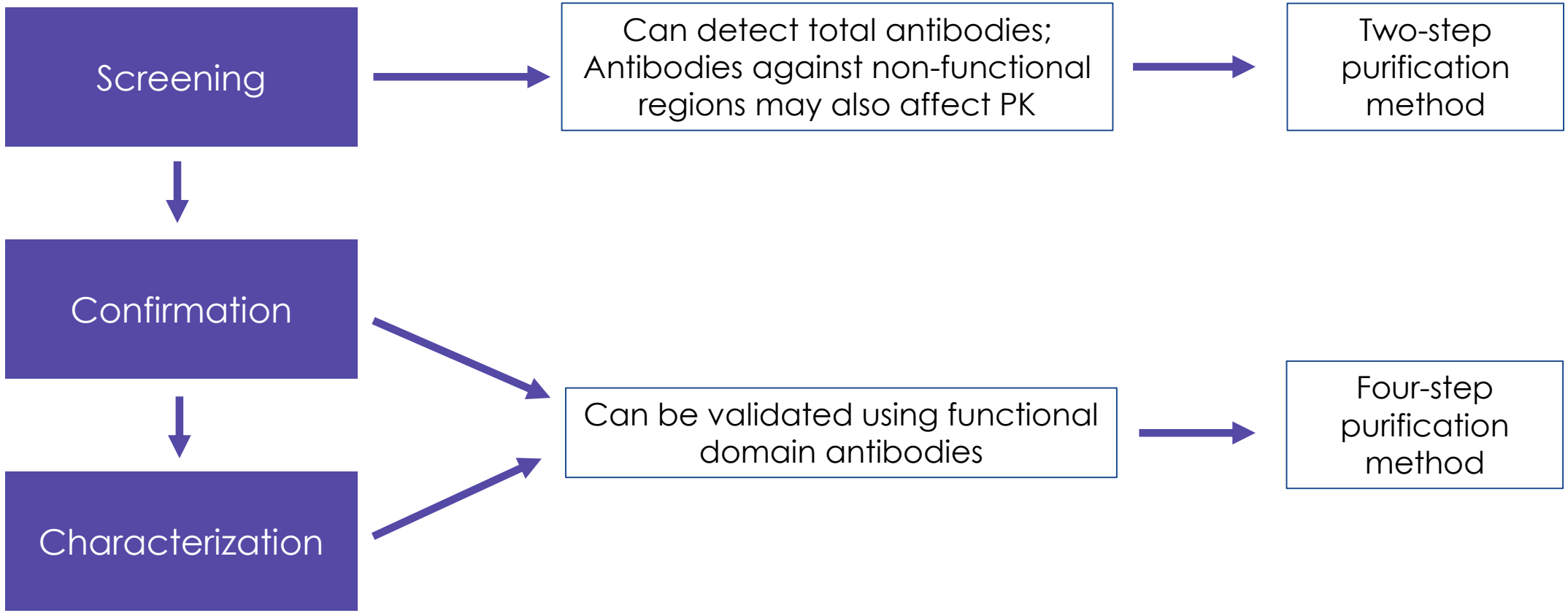
# Anti-ID Polyclonal Antibody Development Process and Difficulty Analysis

1. Anti-ID polyclonal antibody purification



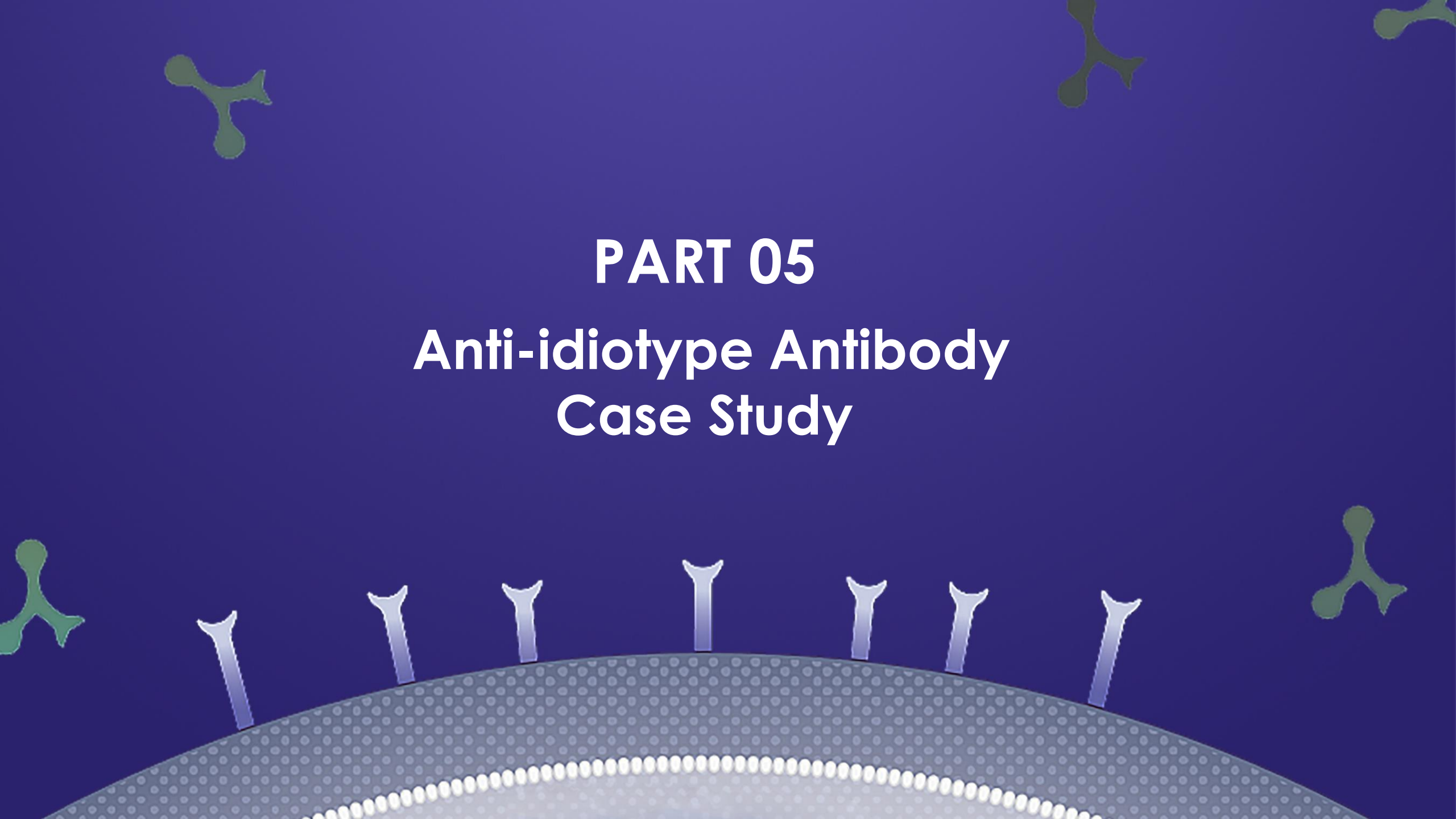
# Anti-ID Polyclonal Antibody Development Process and Difficulty Analysis

2. Selection of different purification methods for anti-ID polyclonal antibodies



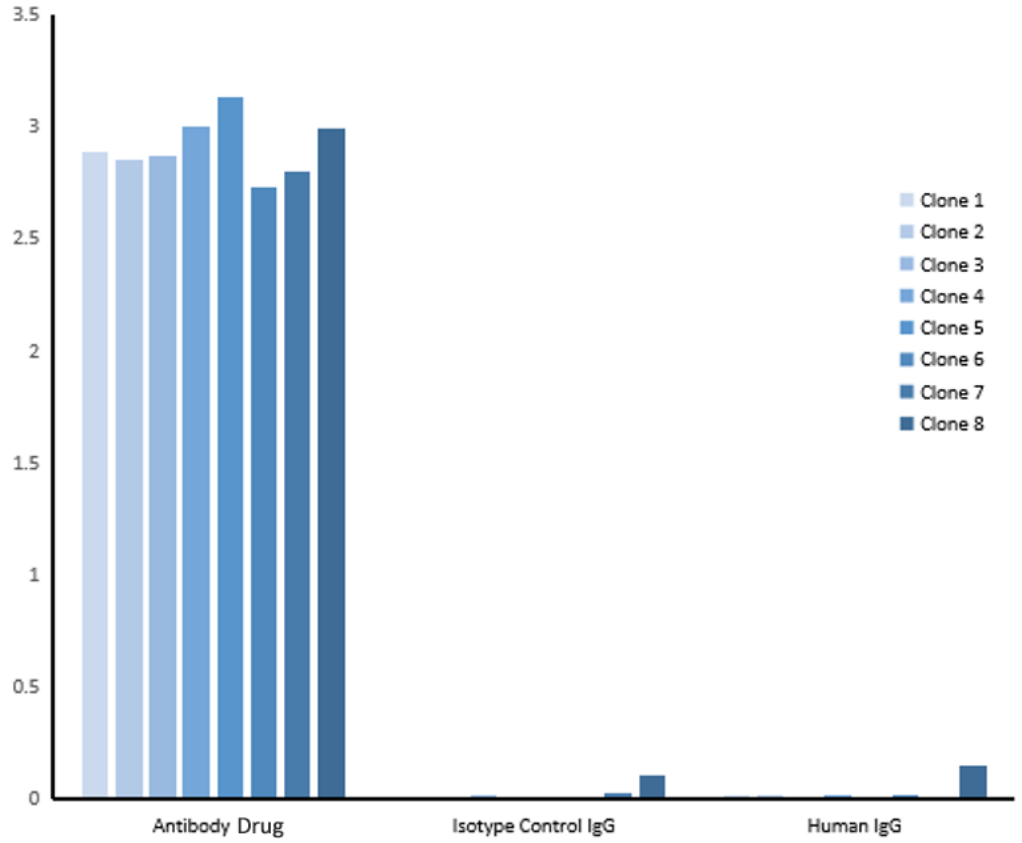
# PART 05

# Anti-idiotype Antibody Case Study

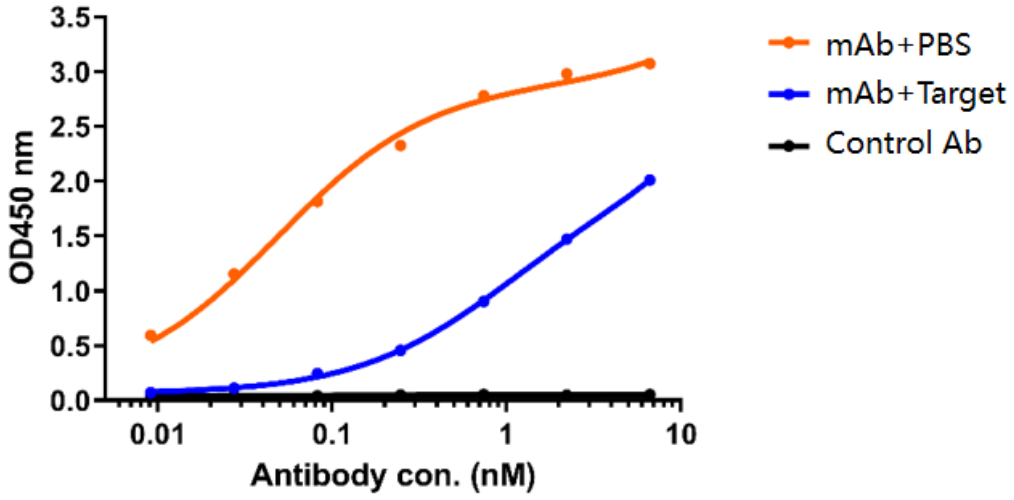


**Example 1**:

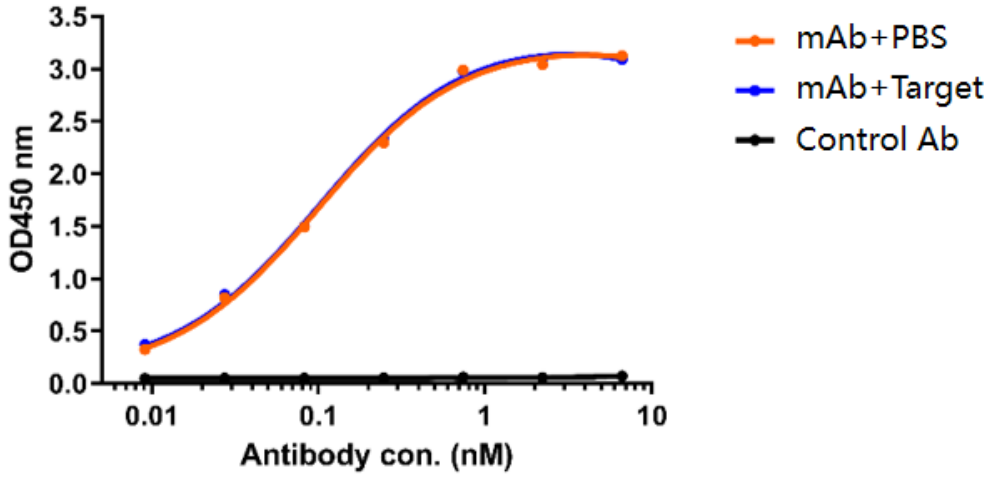
Anti-ID monoclonal antibody has no cross reaction with isotype human IgG and total human IgG



# **Example 2**: Competition ELISA of anti-ID monoclonal antibody

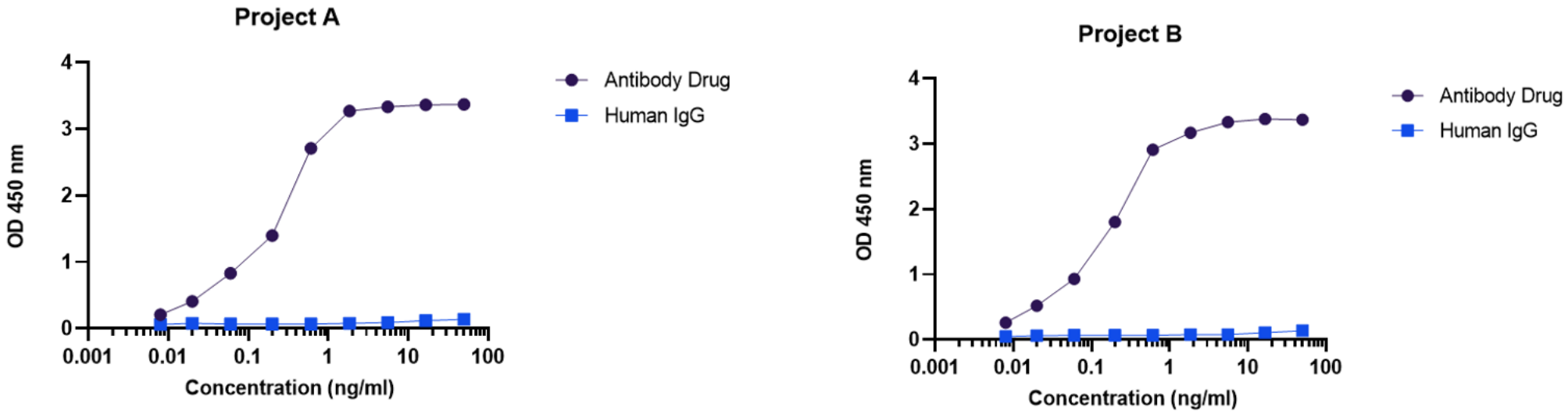



Competitive anti-ID antibodies




Non-competitive anti-ID antibodies

# **Example 3**: Anti-ID polyclonal antibody—good specificity, little cross-reactivity with human IgG



# Anti-ID Antibody Development Services

| Services | Delivery | Lead Time |
|---|---|---|
| Anti-idiotype rabbit polyclonal antibody preparation service | Purified antibody; Antibody QC report | 3~4 months |
| Anti-idiotype mouse monoclonal antibody preparation service | Compete and/or non-compete purified antibodies; Antibody QC report; Hybridoma cell line | 4~6 months |
| Phage displayed library screening of anti-idiotype antibody | Purified antibody; Plasmid; Strain; Antibody sequences, Antibody QC report | 3~6 months |
| Development of immunogenicity detection kits | Kit; Manual; R&D report | 3~4 months |
| Development of PK detection kits | Kit; Manual; R&D report | 3~4 months |

Creative Biolabs®

# Contact Us

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