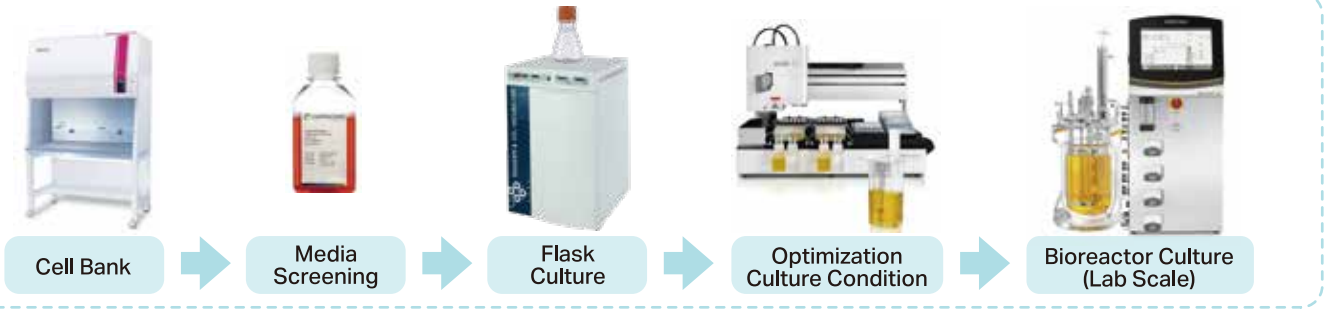
Process Development

We are an Extension of Your Lab

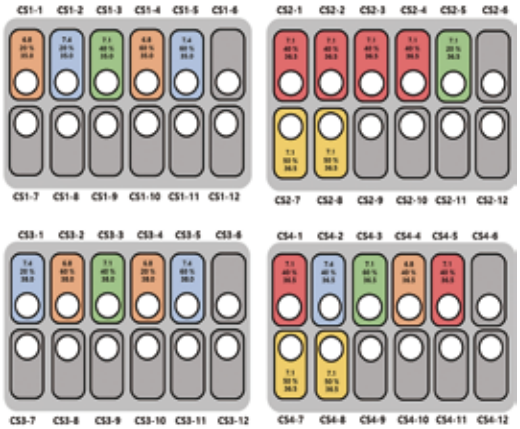
PRESTIGE BIOLOGICS

Upstream Process Development

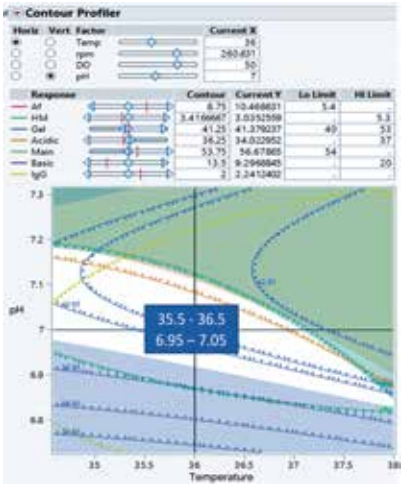


Optimization of Culture condition - Design of Experiments

Ambr®15



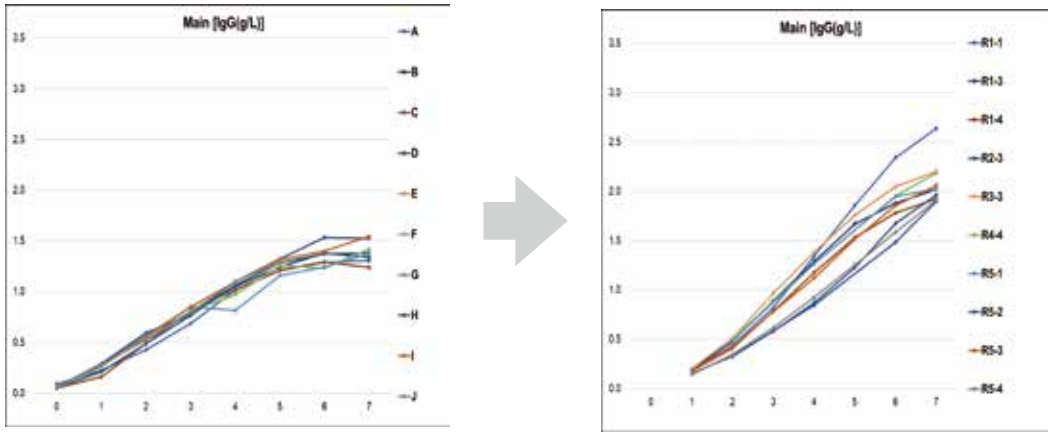
< Experiment Layout >



Experimental conditions were designed through DoE to find the optimization conditions of temperature, pH and DO, which are important process variables, and to set the criteria.

Research accomplishment – Titer improvement

Process development using media developed and Titer is improved due to selection media.



< IgG Titer improvement >

NOTE

The cultivation process development process is carried out as follows. Using the provided cell bank, media screening is conducted to select the optimal media, followed by inoculation in flasks for seed culture. The main culture is then performed using Ambr15, and a Design of Experiments (DoE) utilizing the JMP program is used to set various culture conditions. Through experiments, cell growth, metabolite production, IgG titer, and other factors are analyzed. After the completion of the main culture, the optimal cultivation conditions are determined through quality analysis. The selected optimal conditions are then tested in lab-scale bioreactor cultures to verify if the results align with the DoE analysis. Any necessary process improvements are made to ensure the conditions can be applied at a large scale.

Design of Experiments (DoE) is being utilized to optimize the cultivation conditions. By randomly selecting important process variables such as temperature, pH, and dissolved oxygen (DO), a variety of experimental groups with different culture conditions are established. The DoE experiments are conducted using Ambr15, which enables simultaneous experimentation with multiple culture conditions. Using the JMP program, data on cell growth, metabolite production, IgG titer, and quality analysis are collected for each experimental group, resulting in a Design Space. The obtained Design Space is then used as a reference to set optimal temperature, DO, and other criteria.

One of the achievements in the cultivation process development research is the improvement of IgG titer. A notable example is the enhancement of IgG titer through media screening and the development of optimized media for the process. Prior to using the selected media, the IgG titer ranged from 1.2-1.5 g/L. However, in experiments using the selected media, the IgG titer significantly increased to a range of 1.9-2.6 g/L, more than doubling the titer. The highest achieved IgG titer was 2.6 g/L.

Downstream Process Development

Resin Screening
(AEX, CEX, HIC, Affinity)

Scale down chromatography

Scale down UF/DF

Full DSP Process

Optimization of Culture condition - Design of Experiments

Development and optimization of purification process using the PBL technology of "Non-Protein A purification process for antibodies."

Protein A Process

Product Yield: ≥ 70%, Purity: ≥ 95%

01	02	03	04	05	06
Protein A affinity Chromatography	Viral Inactivation	Intermediate Chromatography	Polishing Chromatography	Virus Filtration	Ultrafiltration / Diafiltration

Non-Protein A Process

Product Yield: ≥ 71%, Purity: ≥ 99%

01	02	03	04	05	06
Ion Exchange Chromatography	Viral Inactivation	Intermediate Chromatography	Polishing Chromatography	Ultrafiltration / Diafiltration	Virus Filtration

Advantage of Non-Protein A process compared to Protein A process

1. Economically efficient due to reduction of unit production price
2. Low risk of resin contamination (1N NaOH can be used for CIP)
3. No issue of residual Protein A leaching

This technology offers a cost-effective purification process for antibody production, eliminating precipitates by adjusting pH and utilizing Ion Exchange, Intermediate, Polishing chromatography columns to efficiently remove impurities like host cell proteins (HCP), without relying on expensive Protein A columns.

NOTE

Purification process development starts with the Resin Screening stage to select high-performance Resin candidates used in the Chromatography process. The selected Resin is then used for chromatography process development. Subsequently, process optimization is carried out through Ultrafiltration/Diafiltration (UF/DF) process development. After process development, the final Drug Substance (DS) is purified through the Full DSP (Downstream Processing) process. This involves conducting quality analysis of the DS to verify if the desired product specifications have been achieved.

Non-Protein A process technology is a purification process technique used to purify high-quality antibody drugs without relying on the Protein A process which is commonly used in protein purification. The Protein A process utilizes affinity chromatography with Protein A resin, which is a surface protein found in the cell wall of Staphylococcus aureus bacteria.

It specifically binds to immunoglobulin G(IgG) antibodies, making it commonly used as a ligand for antibody drug purification. While the Protein A process has the advantage of achieving high purity, it is significantly more expensive (over 20 times higher) compared to general ion exchange resins, resulting in higher production costs. Non-Protein A purification process utilizes ion exchange chromatography instead of the affinity chromatography with Protein A resin during the initial stages to purify high-quality antibody drugs.

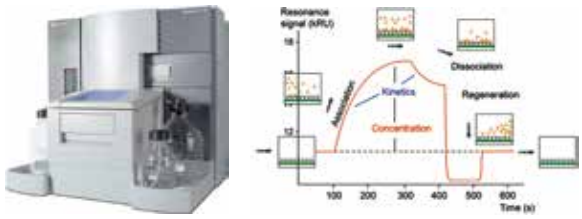
Advantages of Non-Protein A process compared to Protein A process:

1. Cost savings and economic benefits,
2. Lower risk of resin contamination (allowing the use of 1N NaOH for CIP(resin cleaning)).
   : Protein A resin has limited chemical stability, preventing the use of 1N NaOH for cleaning (cleaning is performed with relatively lower concentrations of NaOH). However, ion exchange resin allows the use of 1N NaOH, which enables a higher cleaning effect. Therefore, compared to Protein A resin, ion exchange resin has a relatively lower risk of contamination.
3. No Protein A residue issues.
   : When using Protein A resin for purification, there is a risk of Protein A residues remaining in the process solution, which may pose potential risks when introduced into the human body.

The cost of Non-Protein A resin is approximately 10% of the cost of Protein A resin, therefore, the Non-protein A purification process has price competitiveness.

State-of-the-art analysis Method

1. Kinetics affinity analysis

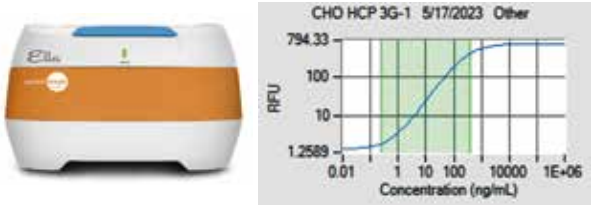


SPR (Surface Plasmon Resonance) analyzer
Test Method : Binding affinity Test

Advantages:
1. Sensitivity, 2. Real-time monitoring, 3. Label-free analysis,
4. High accuracy and reproducibility

Kinetics affinity analysis is performed using Surface Plasmon Resonance (SPR) equipment, which stands for Surface Plasmon Resonance. SPR utilizes a CM5 chip or other surfaces coated with Ligands to immobilize them on a metal surface. When an Analyte is injected, the binding of Ligand and Analyte induces a change in the refractive angle, which can be detected as a response, allowing the measurement of Kinetics and affinity. This analysis method calculates the Kinetics value based on the binding and dissociation rates of antigen-antibody interactions, providing a measurement of affinity strength. SPR offers better reproducibility and less inter-operator variation compared to ELISA, and it provides Kinetics values as numerical data.

2. Automated ELISA analysis

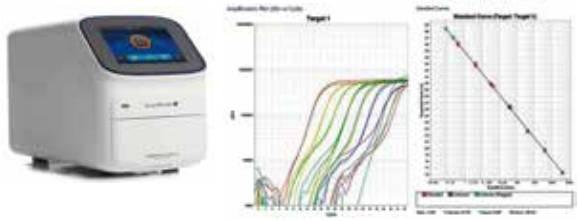


ELISA automation equipment
Test Method : Host Cell Protein Test, ELISA

Advantages:
1. High sensitivity, 2. Automation : Minimizing human error,
3. Speed : Time to result is about 1 hr, 4. Precise : %CV<10%

ELISA (Enzyme-Linked Immunosorbent Assay) is a quantitative method used to measure the amount of antigen protein in a sample by measuring the activity of enzymes bound to antibodies through antigen-antibody reactions. The use of ELISA automation equipment (such as Ella) can replace traditional manual ELISA analysis. While conventional ELISA analysis takes a minimum of about 4 hours, Ella equipment can complete the experiment in approximately 1 hour. Additionally, there is no need to produce standards directly, and only sample dilution is required, minimizing human error with minimal experimental steps. Therefore, with minimal experimental steps, it is possible to obtain results for a large number of samples for process development in a short period of time.

3. Real Time PCR system

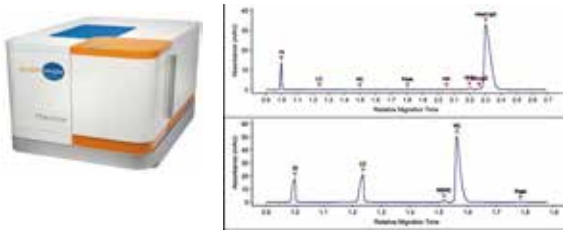


RT-PCR analyzer
Equipment explanation : RT-PCR
Test Method : Host Cell DNA Test, RT-PCR

Advantages:
1. Accuracy and Sensitivity, 2. Flexibility : 96-384 Well plate,
3. Real-time monitoring, 4. Multiplex analysis : Up to 6 Channels

Real-Time PCR is a device that amplifies DNA exponentially and allows real-time monitoring of gene amplification. During each thermal cycle of Polymerase Chain Reaction (PCR), DNA is amplified, and fluorescence is amplified proportionally to the amount of DNA. The amplified fluorescence is tracked in real time to measure the amount of DNA. High reproducibility and sensitivity of Real-Time PCR analysis are utilized to evaluate the quality of Residual DNA in samples, including DS/DP samples, in-process samples, and stability testing samples, for process development.

4. Capillary Electrophoresis analysis



Capillary Electrophoresis equipment
Equipment explanation
: Protein characterization analysis
Test Method : CE-SDS, cIEF

Advantages:
1. High resolution, 2. Real-time monitoring,
3. Automation : Minimizing human error,
4. Faster time to results:
▷ CE-SDS Reduced : 25min, Non-reduced : 35min, ▷ cIEF : 6–10 min

Capillary Electrophoresis (CE) is an experimental technique that combines microcapillary electrophoresis with electrical mobility to separate and analyze small sample volumes. It provides faster results compared to traditional gel-based electrophoresis analysis and it offers real-time monitoring. Purity test (CE-SDS) is conducted by the Maurice equipment which provides higher resolution results and Protein Characterization is conducted by the analysis of charge variants and protein isoforms(cIEF).

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