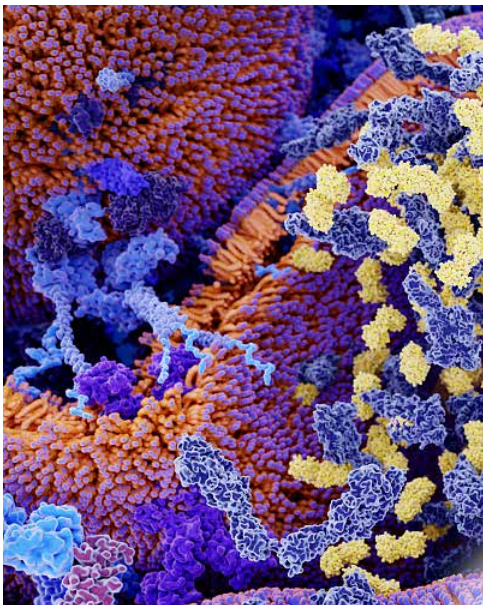
GenScript PROBIO

MULTISPECIFIC ANTIBODY & RECOMBINANT PROTEIN



Development Strategy and Case Study


GenScript
PROBIO

CMC

Multispecific Antibody &

Recombinant Protein

CMC Development Strategy

and Case Study

Multispecific antibody & Recombinant protein CMC Development Strategy and Case Study

GenScript ProBio CMC Track Record

Rich CMC experience with >45 CMC projects
10 IND approvals
52% CMC projects are challenging molecules

Nowadays mAb CMC development is becoming more and more standardized. However, there is no standard CMC strategy for multispecific antibody and recombinant protein. Customized CMC strategy should be developed according to the molecular features. There are many challenges in the development of multispecific antibody and protein CMC, such as low titer, light and heavy chain mismatch, low stability, complex purification process and challenging analytical development, which raise high requirements for CDMOs.

GenScript ProBio has built up a strong process development and analytical development platform for proteins and antibodies. GenScript ProBio has undertaken >45 CMC projects and 52% are proteins and multispecific antibodies. The most advanced project has entered into the clinical phase III. Up until now, GenScript ProBio has successfully helped customers obtain 10 global IND approvals*.

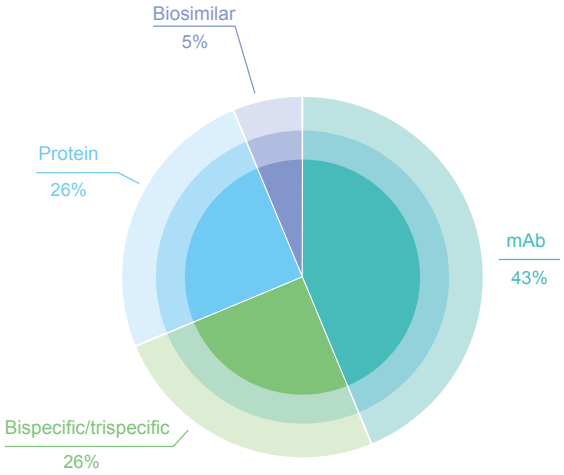


Figure 1. Molecular type distribution of CMC projects undertaken

Progress of Representative Projects

Client	Molecular Type	Stage
Client A	Recombinant protein vaccine	Clinical phase 3
Client B	Cytokine	Clinical phase 2
Client C	Trispecific antibody	IND filing
Client D	Factor	GMP batch delivered
Client E	Cytokine	GMP batch delivered
Client F	Bispecific antibody	GMP manufacturing

*Data as of Nov. 18, 2021

Multispecific Antibody CMC Development

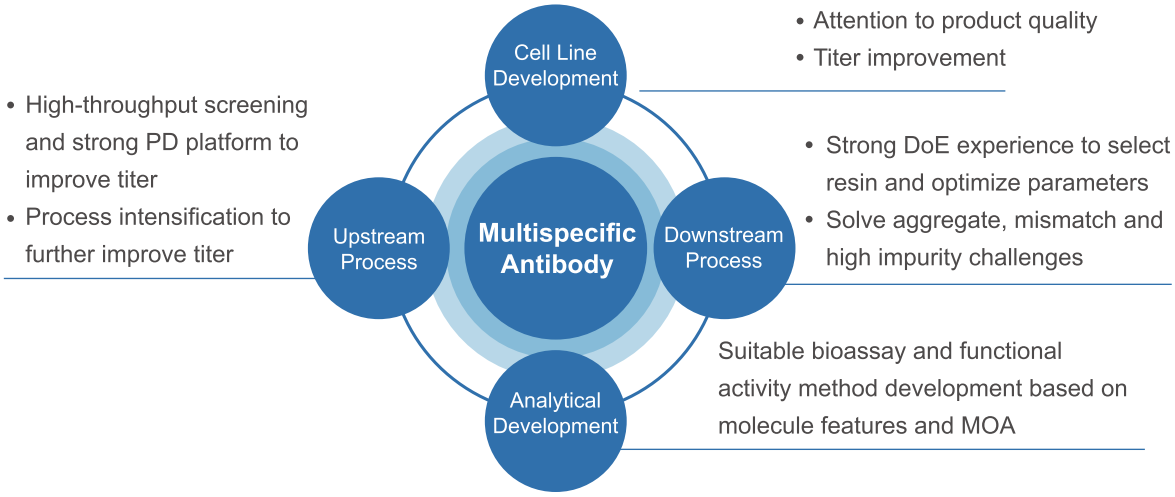


Figure 2. Multispecific antibody CMC development strategy

Cell Line Development—Focus on quality and titer

GenScript ProBio's cell line development platform is based on the proprietary CHOK1-GenS system with high productivity and excellent stability. The average titer for mAb can reach 4.35g/L and the average titer for bsAb is 2.3g/L. It only takes 3.5 months from DNA to PCB.

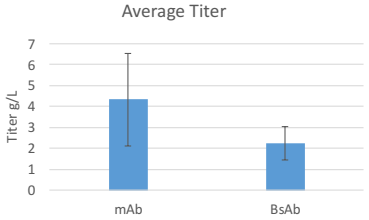


Figure 3. Expression level of CHOK1-GenS

Upstream Process Development

1. Suitable additives to improve product quality

In one bispecific antibody case, modified amino acids help improve titer to 150%.

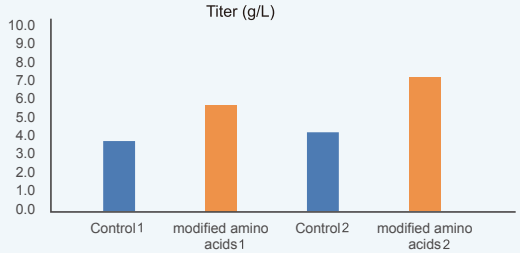


Figure 4. Modified amino acids help improve titer performance

2. Process optimization to improve titer

In one bispecific antibody case, optimizing culture conditions such as pH and temperature can help improve titer performance.

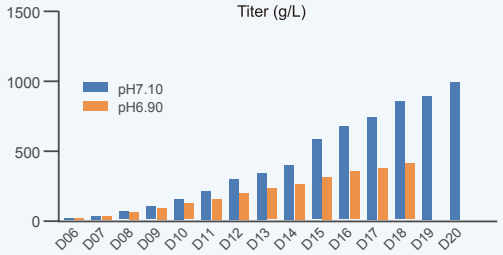


Figure 5. pH optimization helps improve titer performance

3. Process intensification

In addition to the traditional fed-batch process, GenScript ProBio also provides process intensification solutions such as high density inoculation to help improve titer.

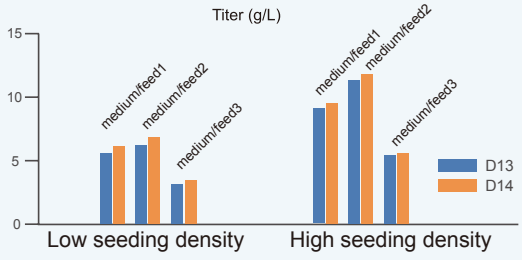


Figure 6. High density inoculation helps improve titer performance

4. Perfusion culture helps improve product quality

Perfusion culture helps increase full length ratio and purity.

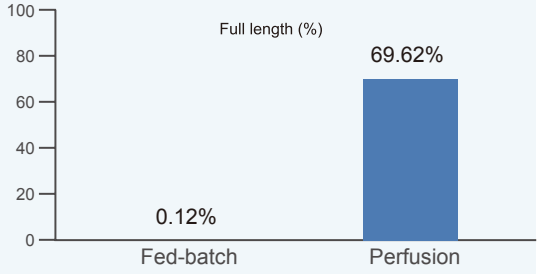


Figure 7. Perfusion process helps improve full length percentage

Downstream Process Development

1. High-throughput downstream process screening platform

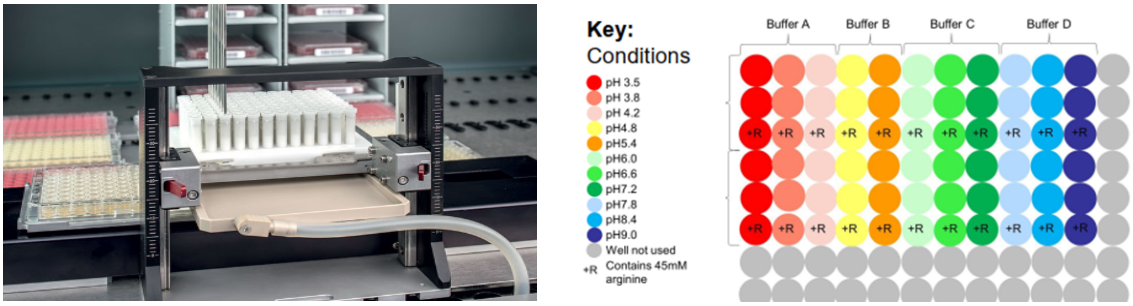


Figure 8. High-throughput downstream platform of GenScript ProBio

2. S/D method in place of low pH can help improve purity

VI Method	Sample ID	Conc.(mg/ml)	SEC-HPLC(%)			CE-SDS-N
			HMW	Main	LMW	(%)
Control	AC Eluate	12.44	1.65	98.35	ND	99.61
S/D	AC Eluate	12.60	1.47	98.54	ND	99.54
Low pH	pH3.8, 1hr	10.73	3.33	96.42	0.24	99.11
	pH3.7, 1hr	10.39	14.61	85.40	ND	99.18

Table 1. Performance comparison between S/D method and low pH

3. Aggregate removal

Multimodal resin is suitable for multispecific antibody purification and can remove aggregates.
In one trispecific antibody case, through downstream process optimization, the purity was improved from 84.7% to 98.6%.

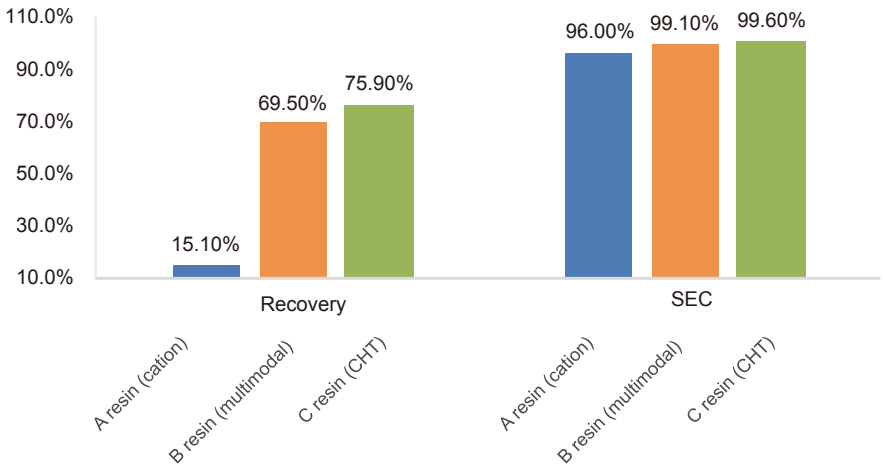


Figure 9. Multimodal resin is suitable for aggregate removal

Analytical Development

Multispecific antibodies often have chain mismatches, so appropriate analytical methods need to be developed to monitor the occurrence of mismatches.
GenScript ProBio has strong analytical development capabilities, and can develop suitable analytical methods and bioassays based on molecule features and MOA. For example, LC-MS method is developed to sensitively monitor chain mismatch.

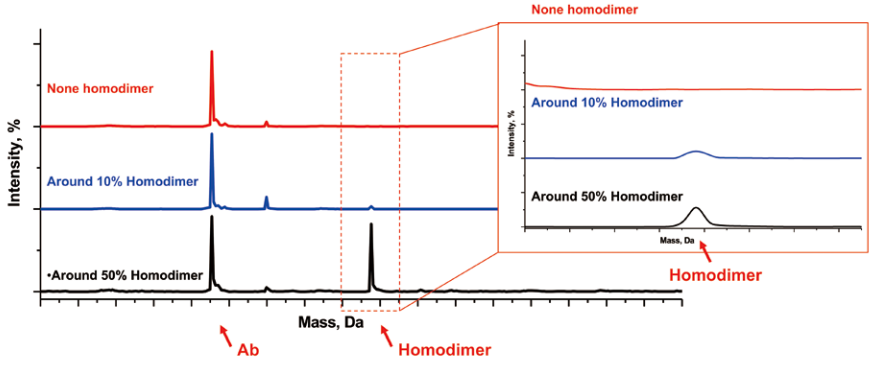


Figure 10. LC-MS method is used to monitor chain mismatch

Case Study:Trispecific Antibody A （Entered into IND filing stage）

Development of asymmetric trispecific antibodies is challenging due to the great difficulties in terms of expression, stability, and bioassay development. GenScript ProBio has successfully delivered a complete trispecific antibody project from DNA to IND for a biotech company in Shanghai, **which has entered into IND filing stage now.**

GenScript ProBio first compared a variety of host cell lines and expression vectors, confirming that GenScript ProBio's proprietary CHOK1-GenS system showed the highest titer. During the cell culture process development, the titer was almost doubled via media screening and additives. In response to abnormal glycan, ion additives helped to lower the level of Man5 by 70%, which can significantly reduce the risk of immunogenicity. In downstream process development, the purity of AC eluate was only 84% with standard platform process. After resin screening, elution parameter optimization and loading capacity optimization, product purity was increased to 96%.

In the bioassay development, it is hard to define suitable *in vitro* bioassay methods to show the real *in vivo* bioactivity due to multiple targets. The bioassay team carried out detailed mechanism investigation to define suitable assays according to the MOA. Finally, the team established the method reflecting the mechanism of T cell engaging based on Jurkat reporter cell line and successfully applied it to the quality control of the product after passing the method validation.

Recombinant Protein CMC Development

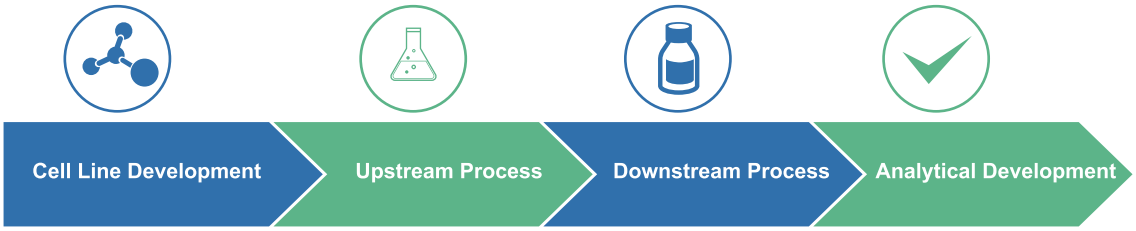


Figure 11. CMC development strategy of recombinant protein

Cell Line Development—Focus on quality and titer

GenScript ProBio's cell line development platform is based on the proprietary CHOK1-GenS system with high productivity and excellent stability. The average titer can reach 4.35g/L. CHOK1-GenS system is compatible with different molecular types, such as bispecific antibody and recombinant protein. For recombinant protein, GenScript ProBio pays attention to product quality and purity, and increase cell pool and clone selection scale to improve final titer.

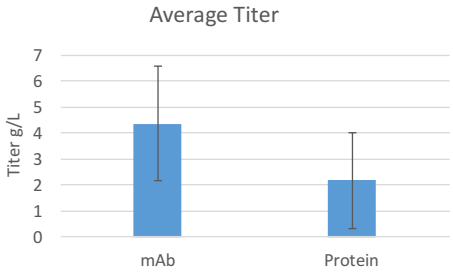


Figure 12. Expression level of CHOK1-GenS

Upstream Process Development

1. Through the use of high-throughput platforms such as Ambr15 for process development, GenScript ProBio can efficiently complete clone screening, media screening and parameter optimization (i.e. pH, temperature, feed strategy). GenScript ProBio optimizes product quality based on molecular features.

Figure 13. Ambr15 micro bioreactor

2. Suitable additives to improve product quality

In one recombinant protein case, ion additives help improve monomer purity.

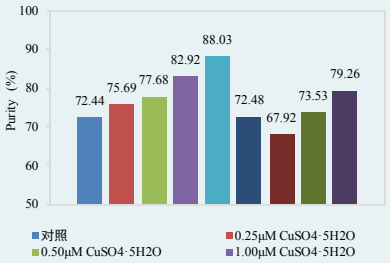


Figure 14. Ion additives help improve product purity

3. Define the best harvesting time

By extending the cell culture time and analyzing the trend of activity and viability, GenScript ProBio defined the best harvesting time as D11.

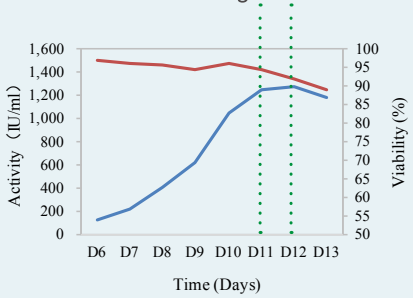


Figure 15. Define the best harvesting time as D11

Downstream Process Development

1. Attention to filter adsorption

There is variant filter adsorption performance in recombinant protein cases. C filter shows the lowest adsorption performance.

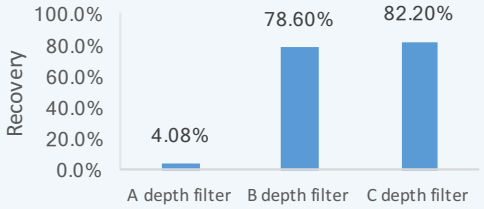


Figure 16. Comparison between different filters

2. S/D substitutes low pH

Protein will fully precipitate under low pH, while S/D inactivation method can act as the substitution. S/D can also help improve the purity due to the aggregate precipitation.

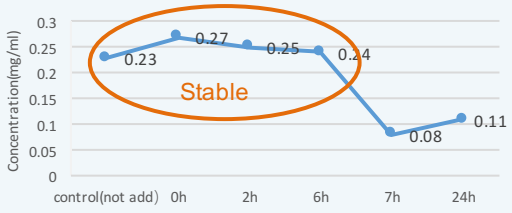


Figure 17. S/D substitutes low pH for VIA

3. AC resin selection

Many recombinant proteins are not suitable for protein A affinity chromatography. Multimodal resin can fulfill the purity and yield requirements.

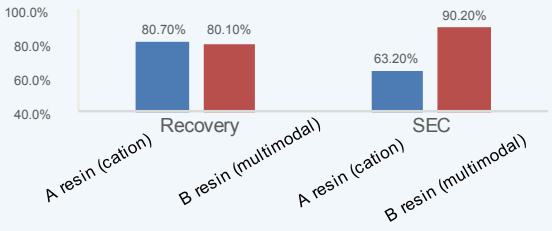


Figure 18. AC resin selection

4.Polish resin selection

Multimodal resin is suitable for recombinant protein polish purification and can remove aggregates.

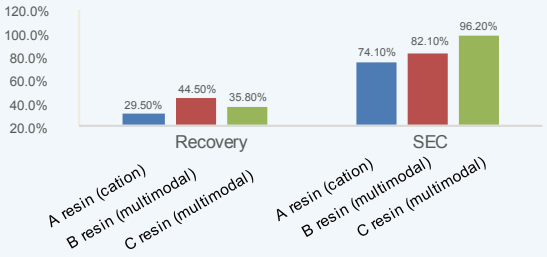


Figure 19. Polish resin selection

Analytical Development

Recombinant proteins have special molecular properties. Conventional analytical methods cannot meet the needs. Strong analytical development platforms, especially in structure, residuals and bioactivity, are essential for recombinant protein projects. This table shows some challenges and solutions in the protein projects done by GenScript ProBio.

Challenge	Solution
Recombinant protein is unstable in mobile phase Standard mAb SEC is not applicable	Mobile phase optimization and selection
Recombinant protein may precipitate during pretreatment Standard mAb glycan analysis is not applicable	Optimize pretreatment buffer Increase sample volume
O-glycan site identification: ESI-MS is not applicable	SialEXO&OpeRATOR LC-MSMS to identify the O-glycan site
O-glycan quantitative analysis: β-elimination method is not stable	LC-MSMS measurement
No commercial Chaperon assay kit No established method	Develop Chaperon assay kit based on ELISA

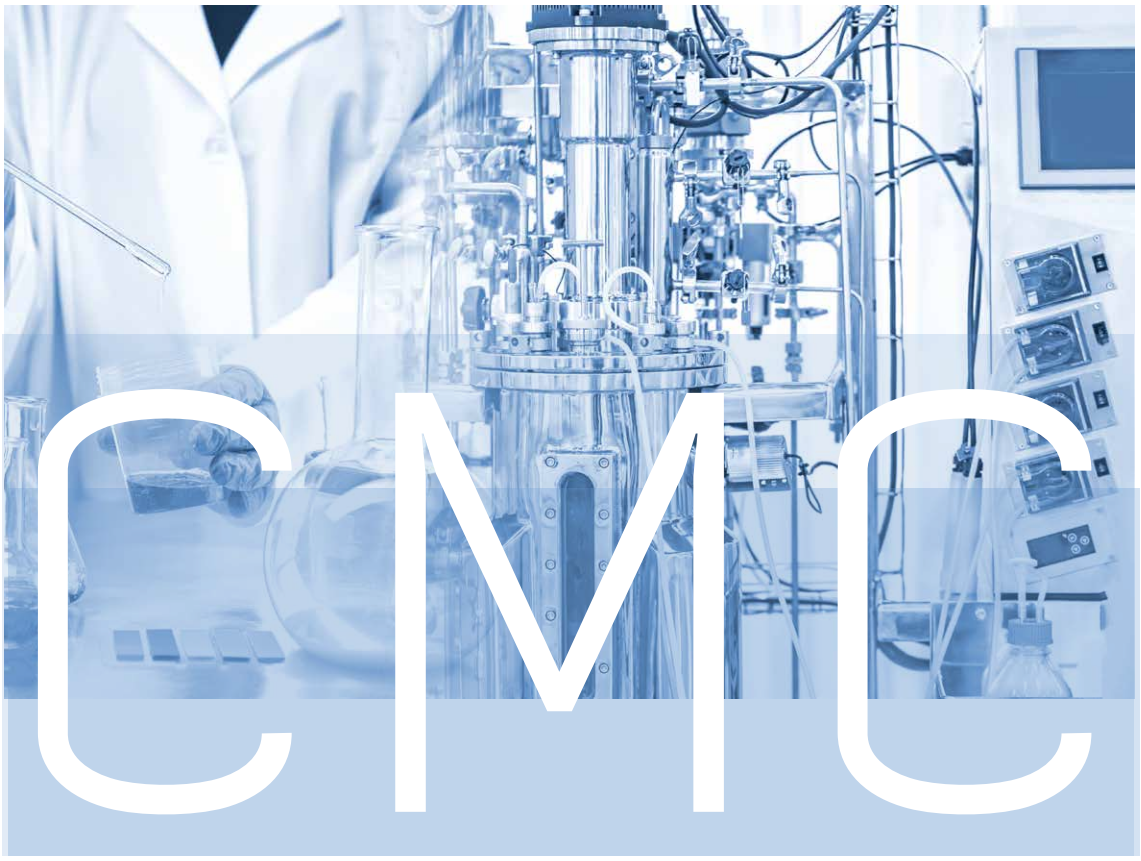
Table 2. Analytical development challenges and GenScript ProBio's solutions in recombinant protein projects

Case Study: Recombinant Protein B (Entered into clinical phase Ⅱ)

In October 2017, GenScript ProBio assisted KLT to obtain NMPA IND approval to carry out the clinical trial of KLT-1101 in the treatment of leukopenia and thrombocytopenia after tumor radiotherapy and chemotherapy in China. GenScript ProBio completed the whole CMC development process from DNA to IND for this innovative biologics.

KLT-1101 is a recombinant human interleukin-12. The interleukin-12 molecule is a complex multi-domain protein with limited titer level. Besides, the characterization and removal of single-domain protein fragments are also difficult in the purification process. Based on years of experience in protein expression and purification, as well as process design capabilities, GenScript ProBio has successfully controlled the aggregates and impurities at a very low level and the titer is 15 times higher than the customer's expectation, which greatly reduces the production cost.

In December 2019, the two parties extended the CDMO partnership to clinical material supply and commercial product supply.



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