Yaohabio
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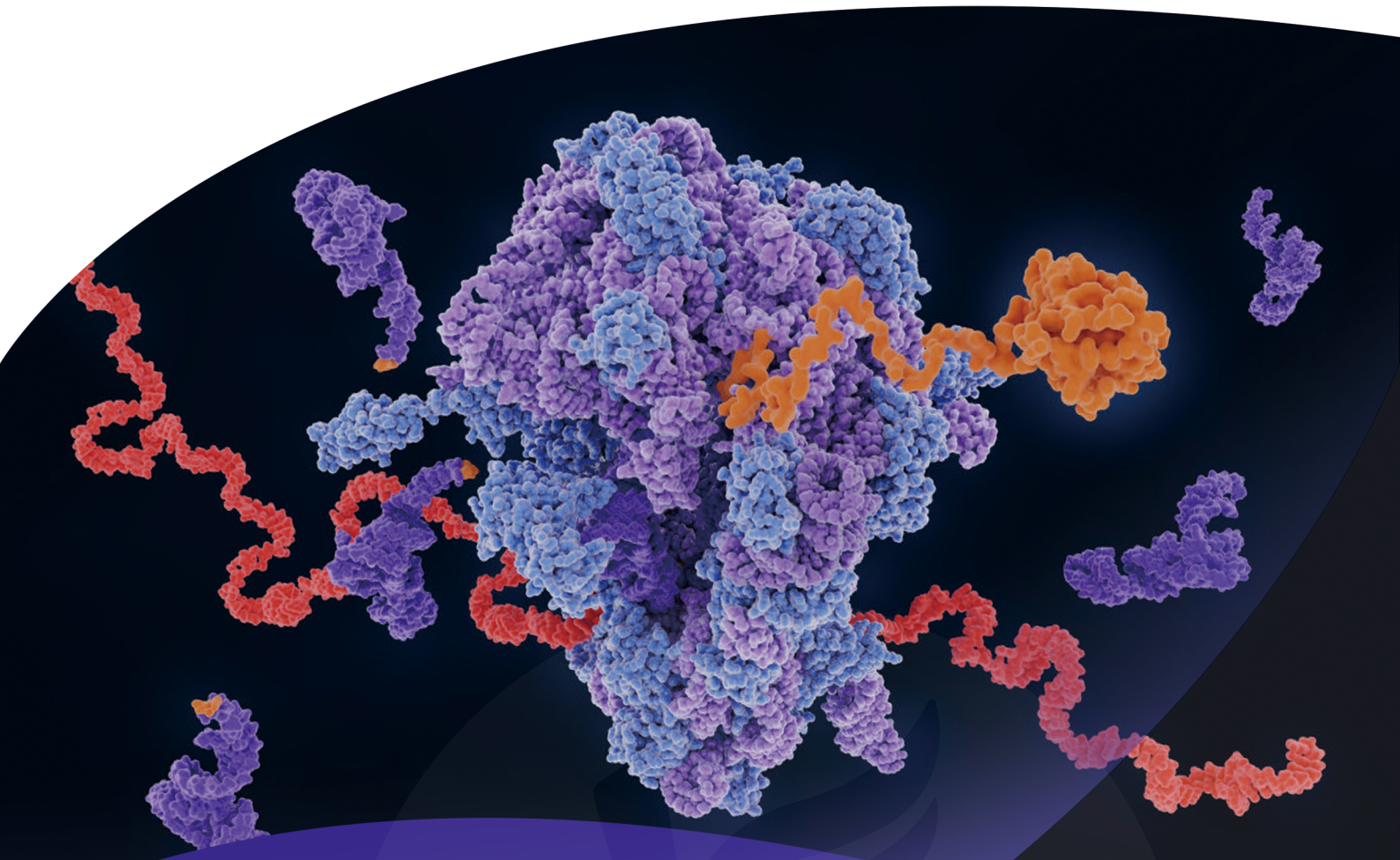
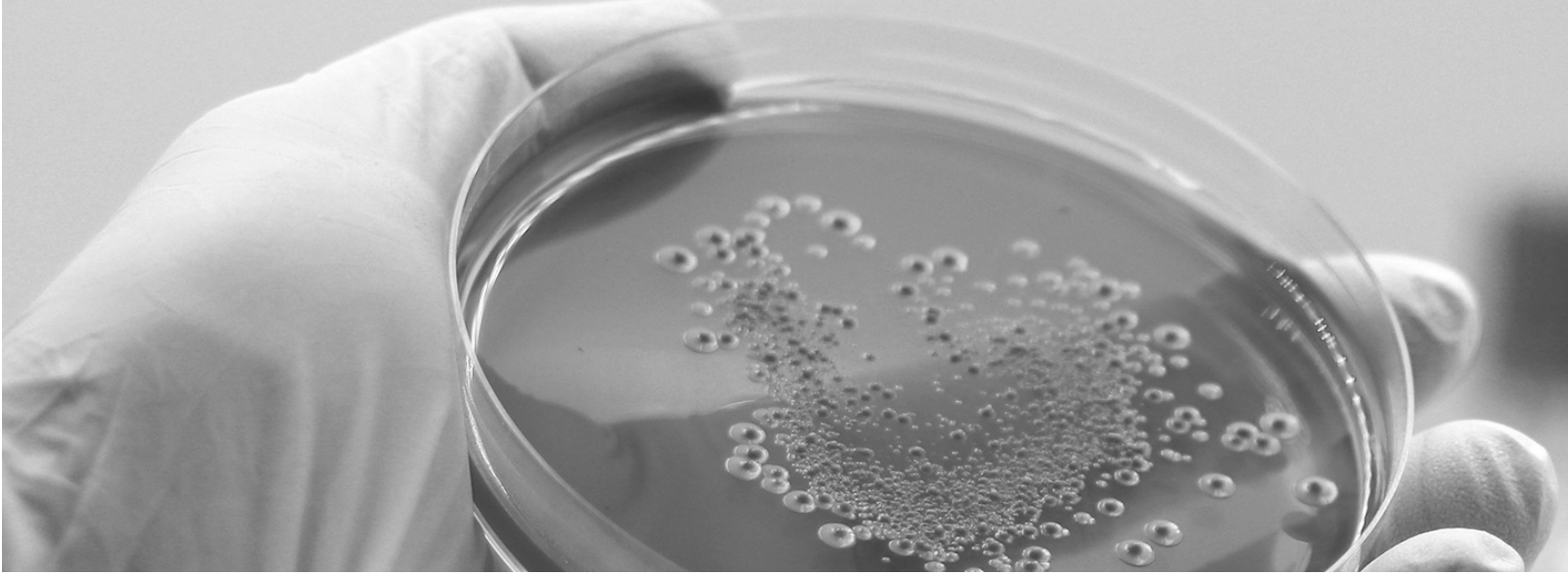
YAOHAIBIO

Recombinant Protein Drug CMO Service Platform



YAOHAI BIO-PHARMA



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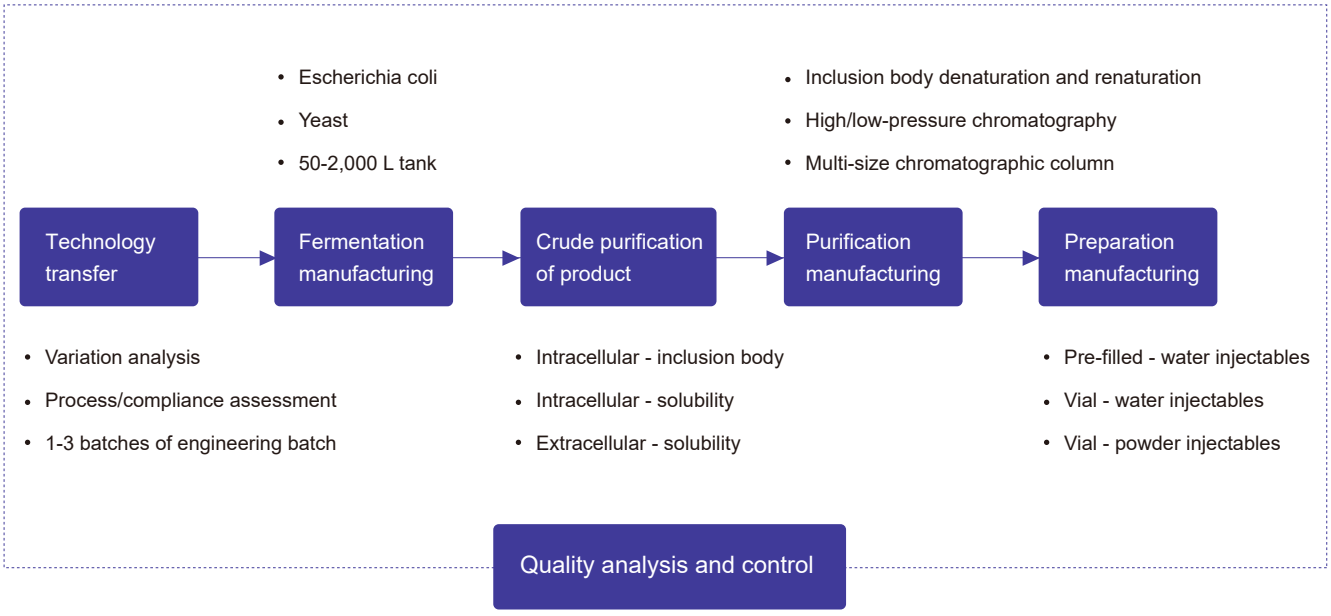
protein drug product CMO service



Yaohai BioPharma has over 10 years of experience in the manufacturing and services of recombinant protein and recombinant plasmid in microbial systems. Leveraging the Escherichia coli and yeast expression system and the GMP level manufacturing workshop, we strictly control the process flow, monitor the release criteria of the raw materials and excipients, intermediate products and final products of the recombinant biological products, and in the meantime make sure the inter-run consistency basing on the comprehensive quality management system to meet the requirements of domestic and foreign laws and regulations in all aspects.

Yaohai BioPharma provides clinical GMP-level recombinant protein manufacturing services, with our platform covering multiple fermentation scales of 50 L-100 L-200 L-500 L-1,000 L-2,000 L matched with low and high pressure chromatography systems of various specifications, as well as the full-automatic filling system for vial water/powder injectables and pre-filled water injectables. We can meet the requirements of manufacturing for IND application samples, phase I-III clinical samples, and commercial manufacturing for Marketing Authorization Holders (MAHs), and will comprehensively accelerate the drug development process.

The recombinant protein products that Yaohai BioPharma CMO platform serve include:

Recombination vaccines

Prophylactic/therapeutic recombinant protein-based vaccines such as virus-like particle vaccine (VLP) and recombinant subunit vaccine.

Recombinant polypeptide

Glucagon-like peptide (GLP-1) analogue, growth hormone (GH), insulin, parathyroid hormone (PTH 1-34, teriparatide) and other polypeptide hormones.

Cytokines

Interleukin-2 (IL-2), IL-15, IL-21, Interferon (IFN), Granulocyte Colony Stimulating Factor (G-CSF), Osteocyte Factor (OF), and etc.

Growth factors

Fibroblast growth factor (FGF), epidermal growth factor (EGF), keratinocyte growth factor (KGF), platelet-derived growth factor (PDGF), and etc.

Enzyme preparation

Cas9 nuclease (gene editing enzyme), other nucleases, tool protease, target protease, etc.

Nanoantibody

Nanoantibody with different potencies (monovalency/bivalency/trivalency).

Collagen

Type III collagen, type I collagen.

Other proteins

Cas protein family, tuberculosis allergen (allergen), antigen, carrier protein, ferritin, human serum albumin fusion protein, MEPE, protein A affinity chromatography ligand protein and other recombinant proteins or polypeptides expressed with E.coli/yeast.

Service details

Service Name	Service Items	Service Details	Minimum Delivery Cycle (working days)	Deliverables
Technology transfer	Document transfer	Manufacturing process/analysis methods/quality specification	TBD	Process transfer report
	Evaluation of technical and regulatory compliance	Man, machine, material, method and environment variation analysis	1	
		Evaluation of formula and process		
		Evaluation of analysis methods	3	
	Protocol transfer	Determination of overall transfer protocol	7	
	Process verification	Manufacturing of 1-3 batches of engineering batch	TBD Subject to customer' s process	
Recombinant protein fermentation manufacturing service	Fermentation pre-production validation	Man, machine, material, method and environment	1	Intermediate products
	Preparation of fermentation system	Preparation of culture medium and solution	2-3	
		Seed tank-fermentor sterilization		
	Fermentation manufacturing	Seed propagation-fermentation-induction	2-4	
		Lowering tank in cooling		
Recombinant protein crude product purification manufacturing service	Pre-production validation	Man, machine, material, method and environment	1	
	Manufacturing preparation	Solution dispensing	1-2	
	Crude purification of product	Culture supernatant collection and concentration- optional	2	
		Bacterial cell collection and crushing-optional	1	
		Inclusion body collection and washing-optional	2	

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Recombinant protein purification manufacturing service	Purification pre-production validation	Man, machine, material, method and environment	1	Protein stock solution
	Preparation of chromatography system	Buffer solution preparation	2-3	
		Filler preconditioning		
	Purification manufacturing	Inclusion body denaturation and renaturation-optional	TBD Subject to customer' s process	
		According to the process: Ultrafiltration, chromatography, enzyme digestion, modification, coupling		
Recombinant protein preparation manufacturing service	Preparation pre-production validation	Man, machine, material, method and environment	1	Vial-water injectables Vial-powder injectables Prefilled syringe-water injectables Cartridge-water injectables
	Preparation pre-production preparation	Apparatus cleaning and sterilization	1-2	
	Preparation manufacturing	Filling of sterilized preparation	TBD Subject to customer' s process	
		Freeze-drying-optional		
		Capping and visual inspection	2	
		Labeling or blind coding	-	

Note:
the mentioned “recombinant protein” generally refers to recombinant protein or recombinant polypeptide; TBD: to be determined (subject to the customer' s process); Multiple testing items can be carried out at the same time.
For CMO project of recombinant protein stock solution + preparation, Yaohai BioPharma' s average delivery cycle is 3-5 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer' s process.

Continued table - quality analysis and control of recombinant protein

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Raw materials and excipients/ packaging materials test and release	Raw materials and excipients-critical items	Conducted in accordance to the specific test items	2
	Raw materials and excipients-full inspection		11
	Packaging materials		60
Recombinant protein quality analysis and control	Appearance, visible foreign material	visual	1
	Insoluble particle	Light obscuration method	1
	Particle diameter	Zeta potential method	2
	pH	Potential method	1
	Total organic carbon (TOC)	UV method	1
	Electrical conductivity	Electrode method	1
	Osmotic pressure molar concentration	Freezing point titration method	1
	Moisture content	Titration method	1
	Loss on drying	Atmospheric pressure/ Vacuum drying method	2
	Residue on ignition	Burning method	2
	Deviation of deliverable volume	Volumetric/gravimetric method	1

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Recombinant protein quality analysis and control	Target protein expression validation	SDS-PAGE, WB, ELISA	2-3
	Target protein expression amount	Non-reducing SDS-PAGE, HPLC, CE	1-3
	Purity of target protein		
	Molecular weight of target protein	Reduced SDS-PAGE	1
	Protein concentration	UV, BCA, Bradford, Lowry	1-2
	Enzyme activity-optional	UV and etc., depending on the characteristics of the enzyme	TBD
	PI isoelectric point	CE	3
	Peptide mapping	HPLC	4
	Bacterial endotoxin residue	Gel method, chromogenic method	3
	Host protein residue-HCP	ELISA	2
	Host DNA residue-HCD	qPCR	1
	Host RNA residue	RT-qPCR	1
	Other customized test items	-	TBD
	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Aseptic test	Direct culture method, membrane filtration method	18
	Investigation of sample stability	High-temperature test	40
		Photostability test	40
		Repeated freeze-thaw test	40
		Accelerated stability test	Sampling: 0, 1, 2, 3 and 6 months
		Long-term stability test	Sampling: 0, 3, 6, 9, 12, 18 and 24 months
GMP workshop environmental monitoring	Non-host strain monitoring	Plate method	5
	Settling microbe monitoring	Culture method	8
	Surface microbial monitoring	Culture method	8
	Planktonic bacteria monitoring	Culture method	8
	Compressed air monitoring	-	10

Note:
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For CMO project of recombinant protein stock solution + preparation, Yaohai BioPharma’ s average delivery cycle is 3-5 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer’ s process.

CMO service features

Multi-scale CMO service platform

The stock solution workshop contains GMP-grade 50 L-100 L-200 L-500 L-1,000 L-2,000 L multi-scale fermentation platform, which is matched with centrifugal, high-pressure homogenization and low-pressure/high-pressure chromatography equipment of corresponding scale. The preparation workshop is accommodated with GMP-level automatic filling systems, covering 1-25 mL vial water injectables (60,000 vials/batch), powder injectables (37,800 vials/batch) and 1-3 mL prefilled syringes/cartridges (20,000 vials/batch).

Standard GMP-level explosion-proof workshop

The explosion-proof solution dispensing system adheres to the explosion-proof requirements. The workshop is equipped with electrostatic discharge instrument and combustible gas alarm devices, which can meet the solution dispensing needs for special processes, such as reverse phase chromatography.

Compliance ensuring platform

Comprehensively evaluate the compliance of products and quality standards, such as host source, antibiotic type, toxicity or sensitization, to meet the requirements of registration application.

Quality control and analysis services

Quality control services driven by the latest edition of Pharmacopoeia and the guiding principles of pharmaceutical manufacturing in China and at abroad, involving the release of raw materials and excipients/packaging materials, intermediate products and final products.

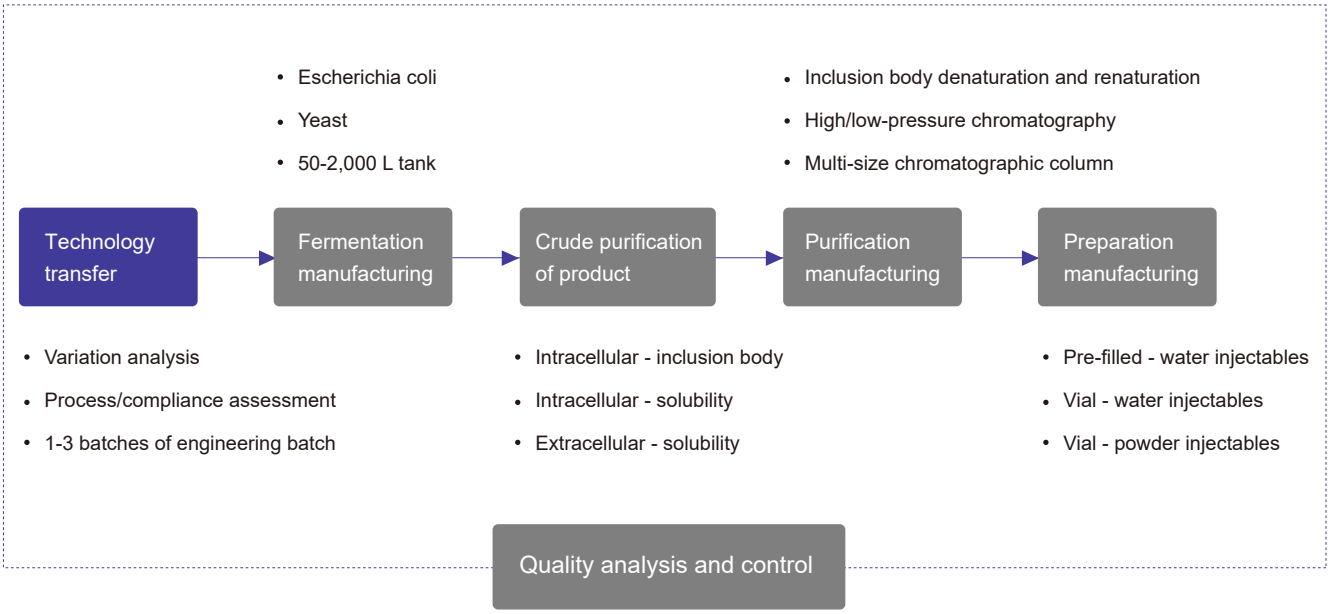
Extensive experience in technology transfer/scaling up

Conversion and scaling up parameters can be adjusted for fermentation and chromatography systems with different scales. More than 100+ recombinant protein-polypeptide-plasmid CMC projects and >5 IND clinical approvals have been successfully delivered, including several China-US dual applications and Australian registered projects.

Open online audit platform

Open online audit port, sharing VR videos of GMP workshop.

Technology transfer



According to the ICH Q10 guidelines, the life cycle of a drug product is divided into 4 stages: drug development, technology transfer, commercial manufacturing, and product discontinuation. Among them, technology transfer is an important part of the drug life cycle and is the key connecting link between drug development and commercial manufacturing. Technology transfer mainly includes manufacturing processes, intermediates control, raw materials and excipients quality criteria, testing methods and other technologies and methods related to product quality. The main goal of technology transfer is to realize the transfer of products and related knowledge between R&D and manufacturing or between different manufacturing sites, including the transfer between MAH and CDMO, CMO as well as CRO enterprises, to realize the sustained and stable manufacturing of products.

Yaohai BioPharma has established a serious of technology transfer management measures ranging from the small test process development, medium test manufacturing to GMP manufacturing stage (stock solution and preparation), and clarified the technology transfer process in accordance with the *Chinese Pharmacopoeia* 2020 edition, ICH Q10, WHO, PDA TR65, ISPE and other technology transfer guidelines. And we have conducted a comprehensive risk evaluation on the transfer process in terms of regulations and quality management and strengthened the management of whole life cycles of drugs based on the concept of Quality by Design (QbD) to ensure the success of technology transfer and fully guarantee the safety, efficacy and quality control of drugs.

01 **Project launch**

- Team building
- Personnel training

02 **Document transfer**

- Manufacturing process
- Quality standard
- Analytical method

03 **Technical assessment Regulatory compliance assessment**

- Man-machine-material-method environment
- Process/formulation assessment
- Test method assessment

04 **Scheme determination**

- Manufacturing conditions
- Sampling plan
- Release criteria

05 **Process validation**

- 1-3 batches
- Engineering batch verification

Service details

Service name	Service items	Service details	Minimum Delivery Cycle (working days)	Deliverables
Recombinant protein manufacturing technology transfer	Document transfer	Manufacturing process	TBD Subject to customer' s process	Process transfer report
		Quality specification		
		Analysis method		
	Evaluation of technical and regulatory compliance	Man, machine, material, method and environment variation analysis	1	
		Evaluation of formulation and process	1	
		Evaluation of analysis methods	3	
	Protocol determination	Transfer protocol determination	7	
	Process verification	Manufacturing of 1-3 batches of engineering batch	TBD Subject to customer' s process	

Note:
TBD: to be determined (subject to the customer' s process).
Reference regulations: *Chinese Pharmacopoeia* 2020 edition; ICH Q10. Guidance for Industry Q10 Pharmaceutical Quality System; WHO Guidelines on the Transfer of Technology in Pharmaceutical Manufacturing;
PDA Technical Report 65: Technology transfer; ISPE Good Practice Guide: Technology Transfer.

Service features

Rich experience in process transfer

fully evaluate the integrity and feasibility of process flow and detection methods, and provide customers with comprehensive process transfer solutions.

Compliance ensuring platform

comprehensively evaluate the compliance of products and quality standards, such as host source, antibiotic type, toxicity or sensitization, to meet the requirements of registration application. Establish the release criteria for raw materials and excipients, packaging materials, intermediates and final products that are compliant, with the whole process complying with the latest version of pharmacopoeia and GMP related guidelines.

Professional project management team

Professional PMs are specialized in fermentation, purification and preparation process transfer and manufacturing process, able to identify and control project risks and drive project operation in whole cycle.

Technology transfer key parameters

Critical equipment	Main reasons affecting process parameters	Key parameters	Yaohai BioPharma equipment
Fermentation tank	Culture volume, diameter-to-height ratio, mixing blade, maximum rotation speed	Aeration, rotation speed, dissolved oxygen	Tofflon, Hanbon
Centrifuge	Sample size, type of equipment (benchtop type, floor type, drum type, disc stack type)	Rotating speed, feeding, residue discharge time	GEA, Beckman, Junmiao
Homogenizer	Equipment brand variation and performance variation	Flow rate, pressure, number of times	GEA, ATS
Chromatography system	UV detector, maximum flow rate	Retention time, sample collection time	Hanbaon, Rongjie
Chromatography columns	Processing batch, column volume	Column volume, loading/buffer solution volume	GE, Hanbon, Rongjie
Filtration/ Ultrafiltration system	Processing batch, membrane area	Membrane area, flow rate	PALL, Sartorius

Note: the column of “Yaohai BioPharma equipment” represents some equipment brands of Yaohai BioPharma. Please consult Yaohai BioPharma’ s staff for more equipment information.

In the process of technology transfer, there are two conversions required. On the one hand, there are different types of equipment requiring conversion of parameters for process transfer or scaling up, such as fermenters, centrifuges and homogenizers. The diameter-to-height ratio, mixing blade distribution and maximum rotating speed of different brands of fermenters were different, while process validation and scale-up can be completed by controlling key parameters such as aeration, rotational speed and dissolved oxygen. In addition, different centrifugation equipment (benchtop type, floor type, drum type, disc stack type) and homogenizers with different performance are suitable for different volumes of samples, so the centrifugal and homogenizing equipment has to be converted for a considerable part of the project process during the scale-up process, including rotational speed, feeding and residue discharge time (disc stack type) for key centrifugation process parameters, and flow rate, pressure and number of times for key homogenization parameters.

On another hand, a conversion is needed only for scale-up parameter when the equipment models are basically the same. For example, during chromatographic purification, with the column height and column efficiency kept within a controlled range and the retention time, loading capacity and elution conditions (linear flow rate) of the original process maintained, only the column volume and sample volume need to be changed according to the actual scale. For filtration or ultrafiltration, it is required to change the membrane area and flow rate according to the actual scale-up scale.

Based on the CMO service experience of 100+ recombinant protein/polypeptide/plasmid, Yaohai BioPharma's team has accumulated experience in equipment-related process transfer of various brands, performances and models. We can quickly identify and adjust key equipment parameters and achieve rapid delivery while maintaining the original quality of customers' products.

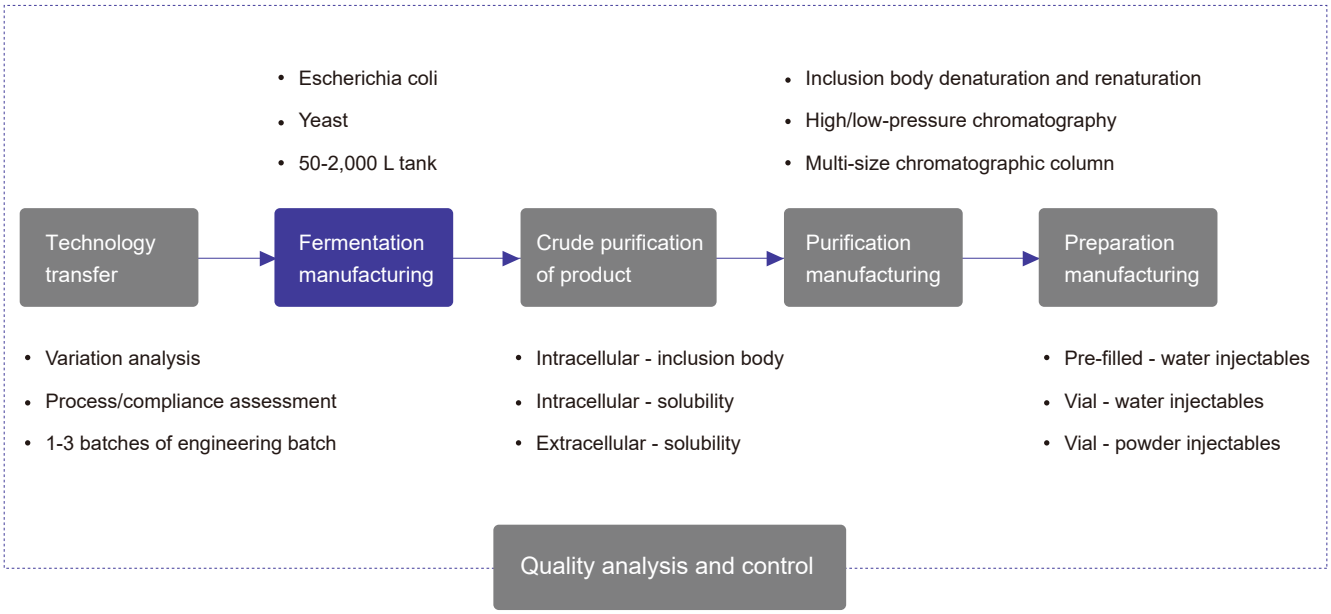
Yaohai BioPharma TIPs: Times of process scale-up is recommended to be within 10 times, and 1-3 batches of engineering batch are recommended to be used to control the risk of process scale-up.

Other Services

- Technology transfer
- Recombinant protein fermentation manufacturing service
- Recombinant protein purification manufacturing service
- Recombinant protein crude purification manufacturing service
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Recombinant protein drug product fermentation

manufacturing service



Yaohai BioPharma has more than 10 years of experience in manufacturing and services of recombinant protein and plasmid in microbial systems, leveraging 5 independently operating GMP-grade customized manufacturing lines with fermentor sizes ranging from 50-140-200-500-1,000-2,000 L to meet different customer needs. We have served 100+ Chinese and foreign customers, with rich experience in industrialized fermentation manufacturing.

During the fermentation process scale-up, process control parameters unrelated to scale are also kept consistent, including culture and induction conditions (such as, basal medium, feeding medium, induction agent, temperature, and pH), and inoculation, feeding and induction. For scale-related parameters, including culture volume, aeration and agitation rate, it is required to control the key parameters during process transfer.

Based on our rich experience in CMO services, Yaohai BioPharma's team can perform the appropriate process transfer and scale-up for different size/brand of fermenters, control key parameters, successfully achieve scale-up manufacturing of upstream processes and transfer to downstream processes with high-quality.

01 Preparation of fermentation system

- Empty elimination of fermentation tank
  Medium - real elimination of fermentation tank
  Preparation and sterilization of other solutions

02 Shake flask culture

- Inoculate working seeds to shake flask for cultivation (Primary or secondary)

03 Seed tank culture

- Seed tank inoculation
  Culture process control

04 Fermentation culture

- Feeding control
  Fermentation process control

05 Induced expression

- Induced expression
  Fermentation process control

Service details

Service items	Service details	Detailed procedures	Minimum lead time (working days)	Deliverables
Recombinant protein fermentation manufacturing service	Fermentation pre-production validation	Man, machine, material, method and environment validation	1	Intermediate products
	Pre-fermentation manufacturing preparation	Receipt of document and material		
		Re-validation of preproduction condition in GMP plant		
	Preparation of fermentation system	Seed tank empty elimination, culture medium preparation and real elimination	2-3	
		Fermenter empty elimination, culture medium preparation and real elimination		
		Feeding tank empty elimination, culture medium preparation and real elimination		
		Preparation of induction agent and antifoam solution		
	Fermentation manufacturing	Seed culture in shake flask	2-4	
		Seed tank culture		
		Fermentation culture		
		Induced expression		
		Lowering tank in cooling		
Line clearance	Line clearance of fermentation workshop	Equipment cleaning and sterilization and environmental disinfection	-	-

Note: the table shows the shortest service period by taking E.coli as an example, and the yeast is increased as appropriate according to the fermentation process.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, complying with all specifications of the latest GMP standards.

Multi-size fermentation system

5 stock solution manufacturing lines, which are built in accordance with international GMP requirements, providing mixing and ventilating fermenters of tank volumes of 50-140-200-500-1,000-2,000 L to support manufacturing needs at different development stages.

Diversified fermentation platform

According to the needs of customers' projects, meeting the needs of the high-density fermentation process, customized feeding and induction process of Escherichia coli and yeast with or without resistance.

Compliant testing and release criteria

Verification of brand and batch number of materials (raw materials and excipients) and release test of key materials will be conducted to ensure consistency and validity of materials.

Single project operation system

Only one project is allowed to run in each workshop in each time period to effectively prevent pollution and mixing, and the next project will be carried out after the line clearance is inspect qualified

Experience sharing of fermentation process scale-up

The key parameters of fermentation process include dissolved oxygen (DO), temperature and pH. Dissolved oxygen is an valid feedback parameter of growth state of strains. Temperature and pH directly affects the growth, proliferation and product expression of strains.

Based on abundant experience in CMO manufacturing services, Yaohai BioPharma summarized the frequently asked questions and scale-up strategies during fermentation process transfer or scale-up.

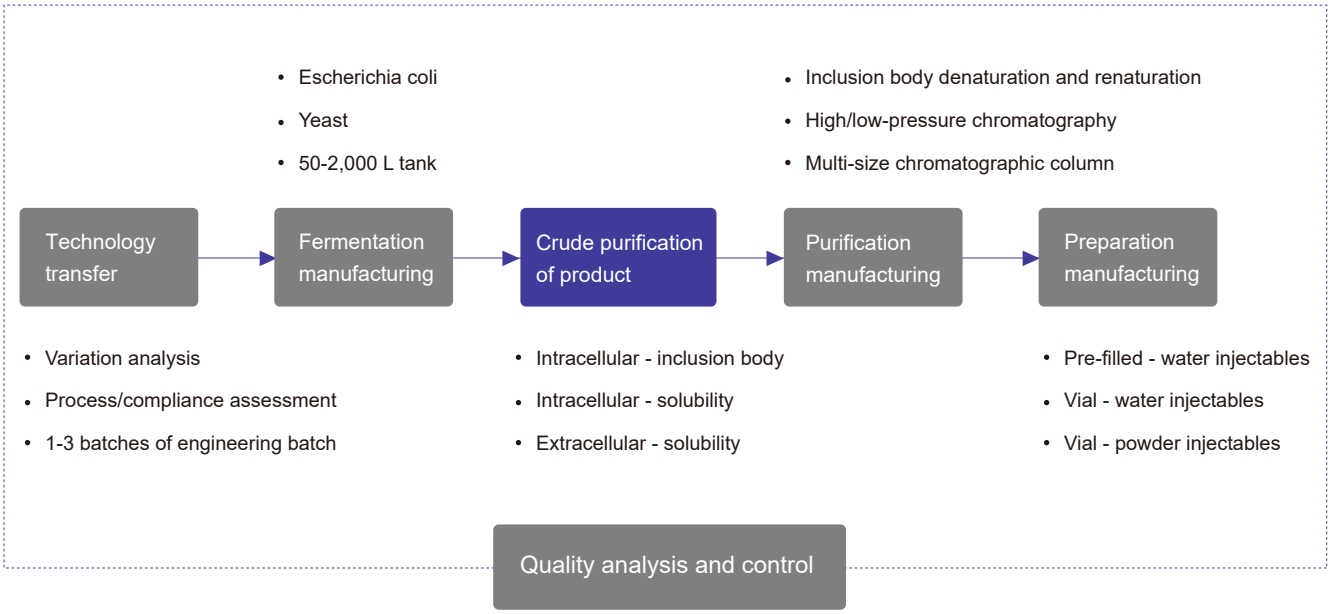
Parameter type	Related parameters	Frequently asked questions	Prevention or solutions
Culture conditions related questions	Temperature and pH	[Volume-independent parameters, consistent]	The temperature, pH sensor, pump and other equipment are calibrated and tested under GMP standards.
	Feeding strategy		
	Induction time		
Bacterial cell volume related questions	Rotation speed	How to conduct process transfer and scale-up if the maximum rotation speed of the fermenter is lower than the original process?	The function of agitation is to mix materials and improve the oxygen transfer coefficient, which is generally adjusted according to dissolved oxygen. A setting of a certain range of rotate speed is recommended during the process development, which may facilitate process transfer and scale-up.
	Ventilation	How to determine the aeration volume of the fermentation process during process transfer or scale-up?	The purpose of aeration is to provide oxygen for bacterial cell, improve oxygen transfer coefficient, and discharge exhaust gas at the same time, and the amount can be set to a fixed value or adjusted according to **dissolved oxygen.** It is recommended that a certain range of aeration amount should be verified during process development to facilitate process transfer and scale-up.
	Dissolved oxygen	The influencing factors of dissolved oxygen include: fermentation liquor volume, viscosity, rotational speed, aeration volume, etc.	The process in which dissolved oxygen can be automatically controlled: after the parameter range of dissolved oxygen is set, it is controlled by adjusting agitation and aeration volume.
	$OD_{600\ nm}$	There is a significant variation between $OD_{600\ nm}$ value and the value of original process	The variation of instruments should be considered. As the principle and sensitivity of different spectrophotometers are different, so it is not recommended to limit OD value excessively.
	Solid content of bacterial solution	-	It is recommended to use wet/dry weight of bacteria cells (weighing method) as the valid parameter of bacteria cell amount in reference to the solid content of bacterial solution (visual method).
	Bacterial cell weight	-	

Tips: it is not recommended to establish quality standards for intermediate products when there are only few running batches. A collection of relevant data is recommended, and then the quality standards and error range can be set by using statistical methods when there are enough data.

Other Services

- Technology transfer
- Recombinant protein fermentation manufacturing service
- Recombinant protein purification manufacturing service
- Recombinant protein crude purification manufacturing service
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Recombinant protein drug product crude purification manufacturing services



Yaohai BioPharma has over 10 years of experience in manufacturing and service of recombinant proteins and recombinant plasmids in microbial systems. Relying on 5 independently operating GMP-grade customized manufacturing lines which are equipped with centrifuges and homogenization equipment of different processing batches to adapt to the tank fermentation batch sizes of 50-140-200-500-1,000-2,000 L, we can meet different customer needs. At present, we have served 100+ Chinese and foreign customers, with rich experience in industrial manufacturing of crude purification.

The function of crude purification is to separate substances with large differences, such as solid-liquid separation, intracellular or extracellular substance separation; the crude purification process with Escherichia coli or yeast as the expression platform includes the separation of culture supernatant, intracellular soluble substances or inclusion bodies, which is usually realized by centrifugation and crushing. Due to the different scale of small tests and manufacturing, the adapted centrifugation and homogenization equipment also varies, and the conversion of centrifugation and homogenization parameters is especially important, which largely affects the quality and yield of the product.

Based on our rich experience in CMO services, Yaohai BioPharma's team can perform the corresponding parameter conversion for centrifuges and homogenizers of different scales/performance, control the key parameters, and successfully realize the scale-up manufacturing of crude purification process with some impurities effectively removed.

01 Solution preparation

- Buffer solution preparation

02 Bacterial cell collection

- Centrifuge to collect bacteria cells
  Rotation speed/feeding residue discharge time

03 Bacterial cell crushing

- Cell heavy suspension and crushing
  Flow rate/number of pressure

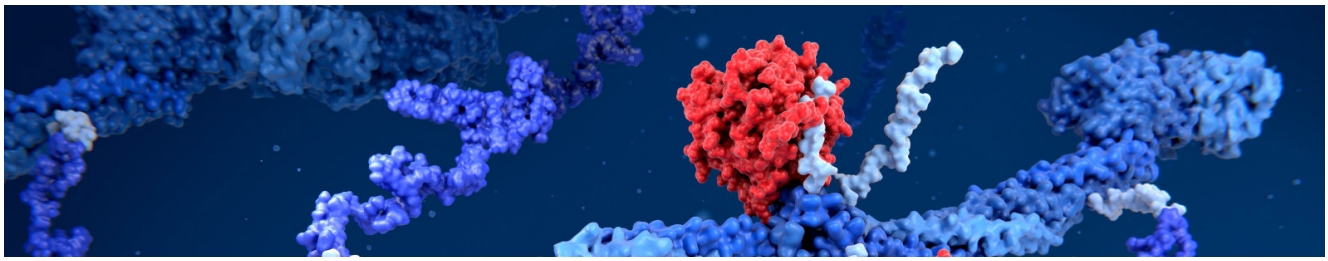
04 Inclusion body collection

- Collection of inclusion bodies by centrifugation
  Rotation speed/feeding/residue discharge time

05 Inclusion body washing

- Washing, centrifuging and dispensing
  Rotation speed/feeding/ residue discharge time

Note:
The figure shows the crude purification process of intracellular -inclusion body. The crude purification process of the other two expression forms is described as follows:
Extracellular solubility: centrifuge to collect supernatant → concentration and solution replacement
Intracelular solublity: centrifuge to collect bacterial cells → high-pressure homogenizaton and crushing → centrifuge to remove debris of bacterial cells



Service details

Service items	Service details	Detailed procedures	Minimum Delivery Cycle (working days)	Deliverables
Recombinant protein crude product purification manufacturing services	Pre-production validation	Man, machine, material, method and environment validation	1	Intermediate products
	Pre-production preparation	Buffer solution preparation	1-2	
	Extracellular soluble form-optional	Supernatant collection by centrifugation	2	
		Concentration and solution replacement		
	Intracellular soluble form-optional	Centrifugal collection of bacteria cells	2	
		High pressure homogenizing and crushing		
		Centrifugal removing of bacterial debris		
	Intracellular inclusion bodies-optional	Centrifugal collection of bacteria cells	3	
		High pressure homogenizing and crushing		
		Centrifugal collection of inclusion bodies		
		Inclusion body washing and subpackage		
Line clearance	Workshop line clearance	Equipment cleaning and sterilization and environmental disinfection	-	-

Note:
centrifuge and homogenize equipment with high adaptability shall be selected according to the batch size of fermentation liquor/process sample.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, complying with all specifications of the latest GMP standards.

Multi-scale crude purification equipment

5 GMP-grade stock solution manufacturing lines, adapted with benchtop/drum/disc-stack centrifuges and high-pressure homogenizers of different performance to meet the crude purification needs of fermentation liquor of different scales.

Diversified crude purification platform

We can provide crude purification service for extracellular soluble products, intracellular soluble products, and inclusion bodies according to customer' s specific process.

Compliant testing and release criteria

Verification of brand and batch number of materials (raw materials and excipients) and release test of key materials will be conducted to ensure consistency and validity of materials.

Single project operation system

Only one project is allowed to run in each workshop in each time period to effectively prevent pollution and mixing, and the next project will be carried out after the line clearance is inspect qualified.

Experience sharing of scale-up of crude purification process

The purpose of centrifugation is to achieve solid-liquid separation. Application scenarios include collection of bacterial cells or supernatant, removal of bacterial cells debris and collection of inclusion body, and etc. Key parameters include rotational speed, feeding rate and residue discharge time. Centrifugation that does not meet the criteria may result in poor solid-liquid separation, which may lead to the decrease in product yield or increasing of the burden of downstream purification.

High-pressure homogenizer can be used to crush cells and release intracellular products. Key parameters include flow rate, homogenization pressure and number of times, and the control indicator is the crushing degree of bacterial cells. Insufficient crushing of bacterial cells will lead to the decrease in the product yield; while excessive crushing will result in the inability to remove the debris of bacterial cells, releasing of too many impurities and increase of the pressure of purification.

Based on our rich experience in product crude purification services, Yaohai BioPharma's team summarized the frequently asked questions and solutions in the transfer process of centrifugation and high pressure homogenization:

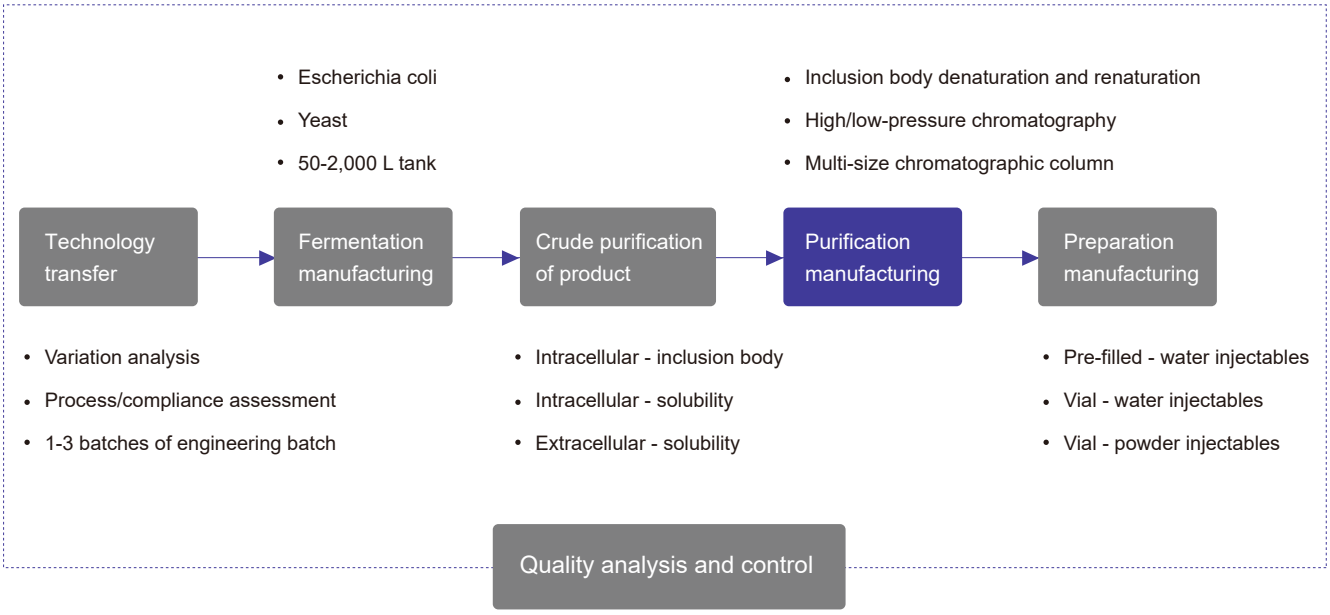
Crude purification process	Frequently asked questions	Question analysis	Solutions
Centrifugation	Turbid supernatant	Poor solid-liquid separation Decrease in yield Increase of purification pressure	• Too much feed: reduce the feeding rate • Uneven feed: fully stirring before feeding • Too low rotate speed: increase the rotate speed • Improper residue discharge time (disc-stack type): adjust the residue discharge time
High pressure crushing	Inadequate crushing of the bacterial cells	Decrease in product yield Increasing of manufacturing cost	• Too much feed: reduce the feeding rate • Low pressure: increase homogenization pressure • Less number of times of homogenization: increase number of times of homogenization
	Excessive crushing of the bacterial cells	Unable to separate the debris of bacterial cells effectively Increasing of the downstream purification pressure May lead to poor product quality	• Higher pressure: lower homogenization pressure • More number of times of homogenization: reduce the number of times of homogenization *Note*: the performance of different brands of homogenizer is inconsistent, so the relevant parameters can not be directly transferred, requiring adjusting of the key parameters. It is recommended to explore a larger parameter range for the small test process to facilitate process transfer.

Other Services

- Technology transfer
- Recombinant protein fermentation manufacturing service
- Recombinant protein purification manufacturing service
- Recombinant protein crude purification manufacturing service
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Recombinant protein drug product

purification manufacturing service



Yaohai BioPharma has over 10 years of experience in manufacturing and services of recombinant protein in microbial systems, leveraging 5 independently operating GMP-grade customized manufacturing lines, which are equipped with tanks of fermentation batches of 50-140-200-500-1,000-2,000 L. The purification workshop is equipped with automatic or manual membrane filtration systems and low/high pressure chromatography systems of different sizes, which can meet the manufacturing needs of filtration clarification, gel filtration chromatography (molecular sieve), affinity chromatography (AC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC, explosion-proof solution dispensing system), composite chromatography, ultrafiltration for solution replacement and etc. At present, we have served 100+ Chinese and foreign customers, with rich experience in purification industrialization manufacturing.

Based on the sophisticated experience in CMO services and diversified chromatography systems, Yaohai BioPharma's team can quickly accomplish the transfer and scale-up of purification process to prepare target products with high purity, high-activity and without toxic residue to meet the release standards in a high-quality way, to meet the downstream preparation manufacturing requirements.

01 **Preparation of purification system**

- Buffer solution preparation
- Filler preconditoning

02 **Inclusion body denaturation and renaturation**

- Form of inclusion body
- Unique process

03 **Filtration clarification**

- Deep filtration
- Remove particles

04 **Chromatography purification**

- High/low-pressure chromatographic column
- Combined purification by multiple chromatography

05 **Concentration and solution replacement**

- Concentration by ultrafiltration
- Concentration by precipitation

Service details

Service items	Service details	Detailed procedures	Minimum lead time (working days)	Deliverables
Recombinant protein purification manufacturing service	Fermentation pre-production validation	Man, machine, material, method and environment validation	1	Recombinant protein-stock solution
	Purification pre-production preparation	Receipt of document and material		
		Re-validation of pre-production condition in GMP plant		
	Purification system preparation	Buffer solution preparation	2	
		Filler preconditioning		
	Purification manufacturing	Inclusion body denaturation and renaturation-optional	TBD (subject to customer's process)	
		Clarification/concentration		
		High/low pressure chromatography		
		Digestion, modification, coupling-optional		
		Concentration and solution replacement		
		Filtration for sterilization		
Line clearance	Workshop line clearance	Equipment cleaning and sterilization and environmental disinfection	-	-

Note:
TBD: to be determined (subject to the customer's process).
The protocol for chromatography are determined based on the process, including but not limited to: gel filtration chromatography (molecular sieve), affinity chromatography (AC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), reverse phase chromatography (RPC, explosion-proof dispensing system), and composite chromatography.

Service features

Mature GMP management system

The workshop staffs and QA/QC personnel have been strictly trained and instructed under GMP, complying with all specifications of the latest GMP standards.

Multi-size fermentation system

5 independent purification manufacturing lines equipped with low-pressure chromatography systems with flow rate of 6-600 L/h, multi-size solution dispensing tanks and chromatography columns, high-pressure chromatographic instrument for industrial preparation, and 5-60 L ultrafiltration system.

Standard GMP-level explosion-proof workshop

The explosion-proof solution dispensing system meets the requirements of explosion-proof, and the workshop is equipped with electrostatic discharge instruments and flammable gas alarm devices, meeting the needs of solution dispensing in special process, such as reversed-phase chromatography.

Compliant testing and release criteria

Verification of brand and batch number of materials (raw materials and excipients) and release test of key materials will be conducted to ensure consistency and validity of materials.

Single project operation system

only one project is allowed to run in each workshop in each time period to effectively prevent pollution and mixing, and the next project will be carried out after the line clearance is inspect qualified.

★★★ To meet the needs of solution dispensing of organic solvent in special processes such as reversed-phase chromatography, Yaohai BioPharma's purification workshops are equipped with explosion-proof solution dispensing systems complying with the requirements of explosion-proof, and are installed with electrostatic discharge instruments and equipped with combustible gas alarm devices.

Experience sharing of purification process scale-up

Filtration clarification is essential for the manufacturing procedure of chromatographic purification. Clarification is designed to further remove particulate substance to avoid negative effects on downstream purification, which is usually done using hollow fiber or membrane packs. Key parameters during process transfer or scale-up include processed batch size, membrane area, and flow rate.

Chromatographic purification is the procedure of removing impurities of different sizes, charges, polarities and specificities using different chromatographic fillers to obtain a high purity target product. The manual/automatic chromatography systems, chromatographic columns and fillers are usually chosen to complete chromatographic purification in manufacturing workshop. Key parameters in process transfer include: processed batch size, column volume, loading volume and flow rate.

Based on our extensive experience in purification manufacturing services, the Yaohai BioPharma's team has summarized the frequently asked questions and scale-up strategies during purification process transfer:

Purification process	Frequently asked questions	Process scale-up strategies
Filtration clarification	What if there is no clarification process, or this process step is omitted in the small tests or medium tests?	*Suggestion*: the samples should be clarified during the process scale-up to remove the solid substances, so as not to increase the burden in the downstream purification.
Chromatographic process	How to scale up the chromatographic process?	*Consistent parameters*: sample concentration and composition, buffer solution composition, filler, column height, linear flow rate, loading volume/ column volume; *Scale-up* parameters: sample volume, column diameter, buffer solution volume, volume flow rate.
Membrane filtration	How to scale up the membrane filtration process?	*Consistent parameters:* sample concentration and composition, membrane pore size, linear flow rate; *Scale-up parameters*: sample volume, membrane area, volume flow rate.
Temperature control of sample-special requirements	If the temperature control of the glass tank is poor, how to improve it?	*Suggestion:* replace with qualified stainless steel tank.

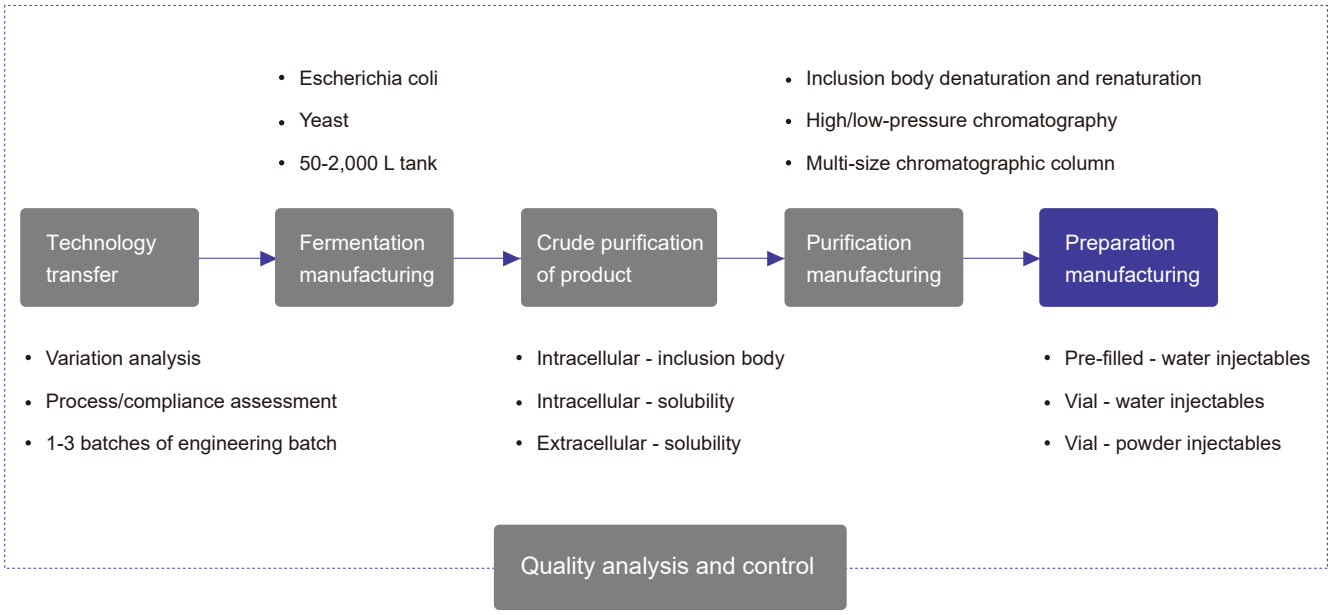
Note: the above table lists some simple and general purification process scale-up strategies. If there are special process needs, you can also communicate with Yaohai BioPharma technical team to solve them.

Other Services

- Technology transfer
- Recombinant protein fermentation manufacturing service
- Recombinant protein purification manufacturing service
- Recombinant protein crude purification manufacturing service
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Recombinant protein drug

preparation manufacturing service



Relying on the GMP-level high-tech automatic manufacturing lines with multiple processes (such as vial washing, drying, sterilizing, aseptic filling, freeze-drying, capping, and etc.) integrated together, Yaohai BioPharma provides the manufacturing services of sterile biopharmaceutical preparations. The types of preparations include vial water injectables, vial powder injectables, and pre-filled water injectables (prefilled syringe and cartridges), with the maximum annual yields of 10 million, 5 million, and 8 million respectively.

Yaohai BioPharma's sterile preparation manufacturing lines conform to the manufacturing specifications for sterile preparations of FDA, EU EMA, China NMPA and Australia TGA, and can be used for the formulation and aseptic filling of drugs and placebos, meeting the needs for IND application, phase I-III clinical research, and MAH commercialization.

Yields / Dosage form	Vial water injectables 1 mL-25 mL	Vial powder injectables 1 mL-25 mL	Pre-filled syringe/cartridge water injectables 1 mL-3 mL
Batch manufacturing	60,000 vials/batch (1-10 mL)	37,800 vials/batch (2 mL/4 mL) 20,043 vials/batch (7 mL/10 mL)	20,000 vials/batch
Annual yields	10 million vials/year	5 million vials/year	8 million vials/year

01 **Preparation for formulation manufacturing**

- Preparation and sterilization of formullation
  Sterilization of rubber stopper-aluminum cap

02 **Vial sorting and vial washing**

- Vial sorting - vial washing
  Drying sterilization

03 **Filling and stoppering**

- Normal/nitrogen filling/ vacuum
  Partial stoppering/full stoppering

04 **Freeze-drying**

- Freeze-drying - full stoppering
  Unique process of powder injectables

05 **Capping and visual inspection**

- Capping - light inspection - warehousing

Service Details

Service items	Service details	Detailed procedures	Minimum delivery cycle (working days)	Deliverables
Recombinant protein preparation manufacturing service	Preparation pre-production validation	Man, machine, material, method and environment validation	1	Vial-water injectables Vial-powder injectables Prefilled syringe-water injectables Cartridge-water injectables
	Pre-production preparation	Receipt of document and material		
		Re-validation of pre-production condition in GMP plant		
	Apparatus preparation	Apparatus cleaning and sterilization	1	
	Preparation manufacturing	Vial sorting and vial washing	1	
		Formulation-optional	1	
		Sample sterilization and filtration		
		Filling and stoppering (normal/nitrogen filling/vacuum)	1	
		Freeze-drying-optional (normal/nitrogen filling/vacuum)	TBD (subject to customer' s process)	
		Capping	1-2	
		visual inspection		
		Labeling and blind coding	-	
Line clearance	Workshop line clearance	Equipment cleaning, sterilization and environmental disinfection	-	

Note: TBD: to be determined (subject to customer's process and batch size);
Vial water injectables/powder injectables, pre-filled water injectables (pre-filled syringe and cartridge) are available for our existing preparation workshops, and communication on other dosage forms are also welcomed.

Service features

Mature GMP training system

Workshop personnel and QA/QC personnel have been strictly training and instructed under GMP, adhering to the specifications of the latest GMP standards.

Diversified preparation types

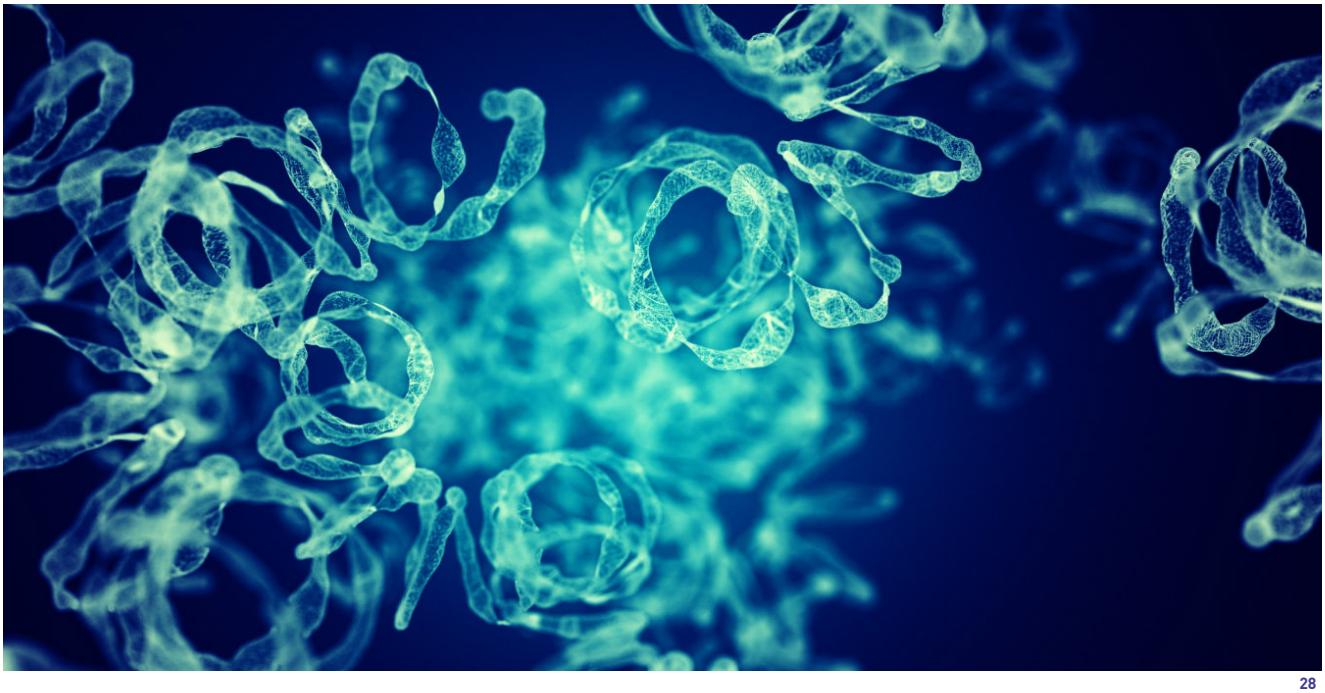
GMP-compliant automated sterile preparation manufacturing lines, serving products including: 1-25 mL vial water injectables/powder injectables, 1-3 mL pre-filled syringe/cartridge water injectables.

Aseptic preparation filling manufacturing line

conforming to aseptic preparation manufacturing specifications of US FDA, EU EMA, China NMPA and Australia TGA. O-rabs system (can be operated to restrict access to isolation system) are used to protect the exposure areas of products (and package materials), providing grade A environmental protection in grade B background.

Rich project experience

100+ CMO project experience; our professional PMs are proficient in preparation process scale-up manufacturing, and can provide professional advice for multiple classes of protein drugs, including the suitability of package materials with drug active substances and excipients, etc.



Experience sharing of aseptic preparation process scale-up

With our extensive experience in preparation filling service, Yaohai BioPharma's technical team can help customers to develop compatible and suitable strategies for packaging materials based on the characteristics of drug solutions and excipients of various types of biological products, and fully promote the manufacturing process of products.

Purification process	Frequently asked questions	Yaohai BioPharma' s experience
Freeze-drying	Why does the freeze-dried powder appear the phenomenon of wall climbing traces of drug solutions?	The phenomenon of wall climbing traces of drug solutions is related to the characteristics of the drug solution (active ingredient and formulation excipients), such as surface activity, surface tension, viscosity, etc.; the different adsorption properties of packaging materials, such as the inner surface of glass bottles, may also lead to the wall climbing traces of drug solutions.
	How to improve if there is the phenomenon of wall climbing traces of freeze-dried powder?	It is recommended to change to glass bottle with lamination without changing the formulation to reduce the adsorbability of glass bottle for drug, so that the phenomenon of wall climbing traces of drug solutions can be improved.

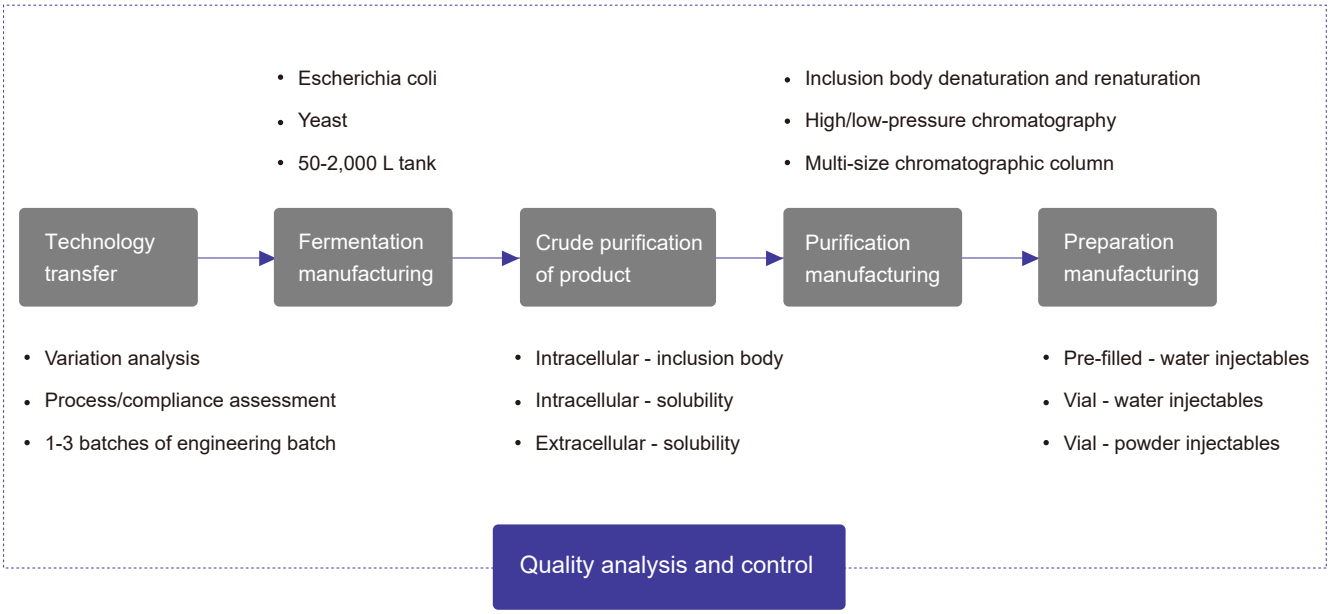
Note:
the phenomenon of wall climbing traces of drug solutions refers to the obvious traces left on the inner wall of the bottle after the freeze-drying of the drug. In normal circumstances, the wettability/contact angle between aqueous solution and low borosilicate glass vial is small, so it is not easy for most varieties of solutions to leave wall climbing traces, and such phenomenon may appear on only a few varieties, for instance, surface active agent is contained in excipients.

Other Services

- Technology transfer
- Recombinant protein fermentation manufacturing service
- Recombinant protein purification manufacturing service
- Recombinant protein crude purification manufacturing service
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Quality analysis and control service

of recombinant protein drugs



According to the pharmacopoeia, the quality control system of recombinant DNA protein products mainly includes raw materials and excipients, package materials, manufacturing process and process control and tests of products. Quality control involves evaluation of known/potential products and process-related substances by using standard substances and verified methods, and analysis of test items of product appearance identification, activity, purity and impurities.

Yaohai BioPharm has a comprehensive quality analysis and control system. Our team members have thoroughly studied and versed in pharmacopoeia and other regulatory specifications, and own rich experience in quality testing and analysis. We are able to implement sample tests in conformity with the specifications, guarantee the conformance to the release criteria of raw materials and excipients, intermediates and stock solutions/preparations, and deliver complete COA reports to customers.

Service details

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Raw materials and excipients/ packaging materials test and release	Raw materials and excipients-critical items	Conducted in accordance to the specific test items	2
	Raw materials-full inspection		11
	Packaging materials		60
Recombinant protein quality analysis and control	Appearance, visible foreign material	visual	1
	Insoluble particle	Light obscuration method	1
	Particle diameter	Zeta potential method	2
	pH	Potential method	1
	Total organic carbon (TOC)	UV method	1
	Electrical conductivity	Electrode method	1
	Osmotic pressure molar concentration	Freezing point titration method	1
	Moisture content	Titration method	1
	Loss on drying	Atmospheric pressure/ Vacuum drying method	2
	Residue on ignition	Burning method	2
	Deviation of deliverable volume	Volumetric/gravimetric method	1
	Target protein expression validation	SDS-PAGE, WB, ELISA	2-3
	Protein expression amount	Non-reducing SDS-PAGE, HPLC, CE	1-3
	Purity of protein		
	Protein molecular weight	Reduced SDS-PAGE	1
	Protein concentration	UV, BCA, Bradford, Lowry	1-2
	Enzyme activity-optional	UV and etc., depending on the characteristics of the enzyme	TBD
	Isoelectric point (pI)	CE	3
	Peptide mapping	HPLC	4
	Bacterial endotoxin residue	Gel method, chromogenic method	3
	Host protein residue-HCP	ELISA	2
	Host DNA residue-HCD	qPCR	1
	Host RNA residue	RT-qPCR	1
	Other customized test items	-	TBD

Service items	Test items	Test methods	Minimum Delivery Cycle (working days)
Recombinant protein quality analysis and control	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Aseptic testing	Direct culture method, membrane filtration method	18
	Investigation of sample stability	High-temperature test	40
		Photostability test	40
		Repeated freeze-thaw test	40
		Accelerated stability test	Sampling: 0, 1, 2, 3 and 6 months
		Long-term stability test	Sampling: 0, 3, 6, 9, 12, 18 and 24 months
GMP workshop environmental monitoring	Non-host bacteria monitoring	Plate method	5
	Settling microbe monitoring	Culture method	8
	Surface microbial monitoring	Culture method	8
	Planktonic bacteria monitoring	Culture method	8
	Compressed air monitoring	-	10

Note:
The mentioned “recombinant protein” generally refers to recombinant proteins or recombinant polypeptides; TBD: to be determined (subject to the customer’ s process). Multiple test items can be carried out at the same time.
For CMO project of recombinant protein stock solution + preparation, Yaohai BioPharma’ s average delivery cycle is 3-5 months (including engineering batch, cycle for reference), and the actual delivery cycle is subject to the customer’ s process.

CMO service features

Mature GMP training system

QA/QC personnel have been strictly trained and instructed under GMP, complying with all specifications of the latest GMP standards.

Compliant QC test process

Being able to reasonably evaluate the compliance of analysis methods and quality release standards, and can quickly complete the transfer and verification of the analysis methods.

Whole-process quality control

Releasing tests of raw materials and excipients, packaging materials, intermediates, recombinant protein stock solutions and preparations will be performed, with the quality criteria of materials and samples strictly controlled.

Complete quality analysis platform

Based on our rich experience in CMO services, Yaohai BioPharma's quality control team has established a highly applicable, robust and reliable analysis platform that can meet the requirements of physiological, biochemical and microbiological testing.

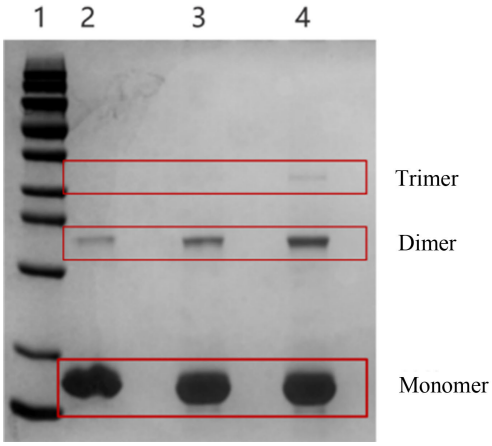
BSL-2 microbiology laboratory certification

Meeting the needs of special projects such as pathogen tests.

Quality analysis case sharing

Improper pretreatment method of the test samples may exert a great impact on the quality test results. In a purity test of recombinant protein freeze-drying powder, different volumes of heavy suspension were used for re-dissolution to obtain protein samples of different concentrations: 1 mg/mL, 5 mg/mL and 10 mg/mL, and non-reduced SDS-PAGE was selected to determine the purity of protein monomer. The results of electrophoresis showed that the content of protein monomers at different protein concentrations varied significantly, so the pretreatment method directly affected the quality index of the product.

Lane 1: Marker;
Lane 2: protein concentration is 1 mg/mL;
Lane 3: protein concentration is 5 mg/mL;
Lane 4: protein concentration is 10 mg/mL
Result analysis: the target protein is known to be hydrophobic and the target product is monomer. The high protein concentration after re-dissolution promotes the formation of protein aggregates, resulting in the reduction of the purity of monomer protein.
[Note: protein loading volume >10 μg (Coomassie Brilliant Blue Staining Method), in accordance with the provisions of the Chinese Pharmacopoeia]



Based on our rich CMO service experience, Yaohai BioPharma's quality control team has established a highly applicable, robust and reliable analysis platform, by which the compliance evaluation, method transfer and verification of quality test methods can be accomplished, to match against the product quality requirements in a high-standard way.

Other Services

- Technology transfer
- Recombinant protein fermentation manufacturing service
- Recombinant protein purification manufacturing service
- Recombinant protein crude purification manufacturing service
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Yaohai BioPharma's GMP

workshop and equipment

Fluid Distribution System

Fermentation System

Disk Centrifuge

Tube Centrifuge

Homogenizer

Hollow Fiber System

Low Pressure Chromatography System

High Pressure Chromatography System

Sterile Filling System

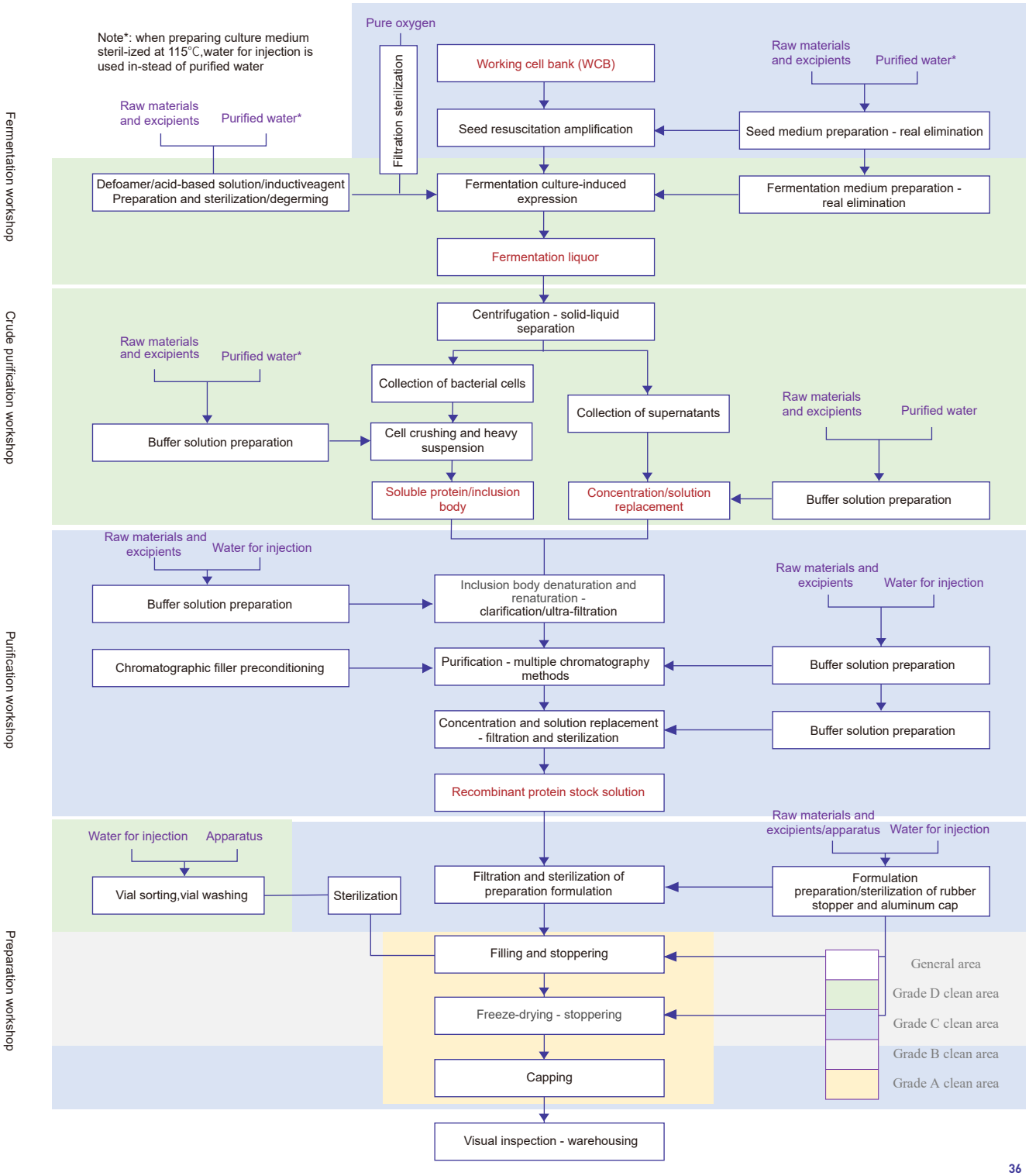
Capillary Electrophoresis Apparatus

Gas Chromatograph

Liquid chromatograph

Functional area classification

of Yaohai BioPharma GMP workshop

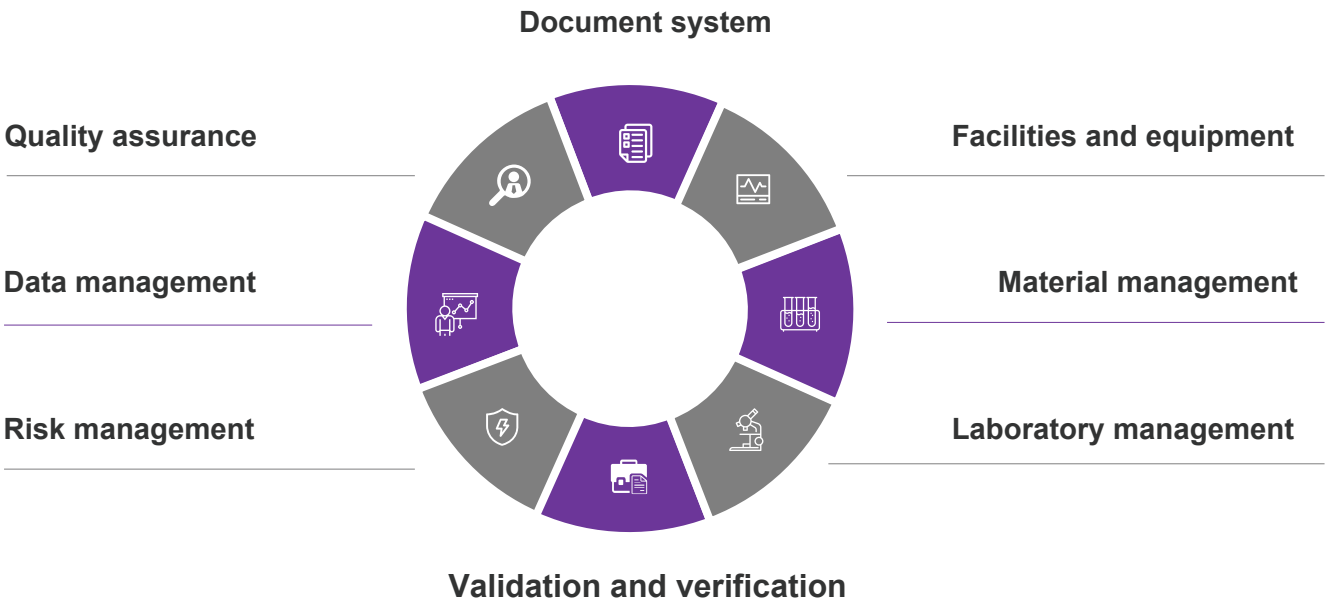


Yaohai BioPharma

GMP quality system

Good Manufacturing Practice (GMP) is the basic guideline for drug manufacturing and quality management, which applies to the whole process of drug preparation manufacturing and the key processes affecting the quality of finished products in API manufacturing. The vigorous implementation of GMP is to avoid contamination and cross-contamination in the drug product manufacturing process to the maximum extent possible and to reduce the occurrence of various errors, which is an important measure to improve the quality of drug products.

Yaohai BioPharma's quality system management personnel have GMP certification experience, and the executive team staff have GMP enterprise work experience. Our team members are proficient in studying, interpreting and translating domestic and foreign regulations, and have developed a compliant quality management system by combining different stages of the life cycle of drugs, to control the whole process of man-machine-material-method-environment management in the manufacturing stages.



Document system

- Policies of management (POL), standard operation procedures (SOPs)
- Process procedures/quality standards/standard test procedures (STP)
- Form records: adhere to SOP and STP, with independent approval

Quality assurance

- System management: document/record, training, change/deviation/ CAPA/complaints, self-test, material/supplier management
- Site management: manufacturing site, QC site, material control, utility system, record review, product release

Data management

- Computerized system management
- Laboratory raw data management
- Data audit, data reliability management

Risk management

- Line confluence risk control: stage manufacturing/dedicated apparatus
- Sterile contamination risk control: facility/equipment/material control
- Compliance risk control: self-test/audit/regulation translating
- Quality system risk control: change/deviation/CAPA

Validation and verification

- Validation of plant and facilities
- Equipment validation
- Computerized system verification
- Process verification
- Metrology management
- Clearance validation
- Aseptic process simulation
- Validity period verification, etc.

Laboratory management

- Management of samples/references, reagents and consumables
- Validation and verification of analysis methods, management of entrusted inspection
- Data, record and report management, quality information management

Material management

- 1,400 m^2 storage area, conforming to GMP and FDA specifications
- For storage of raw materials and excipients, packaging materials, intermediate products, finished products, and etc.
- Storage conditions include freezing, refrigerating or ambient/room temperature

Facilities and equipment

- Management of functional areas of different cleanliness classes: air conditioners are independently formulated to control differential pressure, temperature and humidity and suspended particles
- Safeguard of medium: water for injection, purified water, pure steam, and etc.
- Equipment: authority setting, online monitoring, verification and measurement

Management and control of clean area in GMP workshop

Maximum allowable number of suspended particles/m^3

Cleanliness level	Static		Dynamic	
	≥0.5 μm	≥5.0 μm	≥0.5 μm	≥5.0 μm
Grade A	3,520	20	3,520	20
Grade B	3,520	29	352,000	2,900
Grade C	352,000	2,900	3,520,000	29,000
Grade D	3,520,000	29,000	No provision	No provision

SERVE
WITH HEART &
CREATE
THE FUTURE TOGETHER

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