Yaohabio
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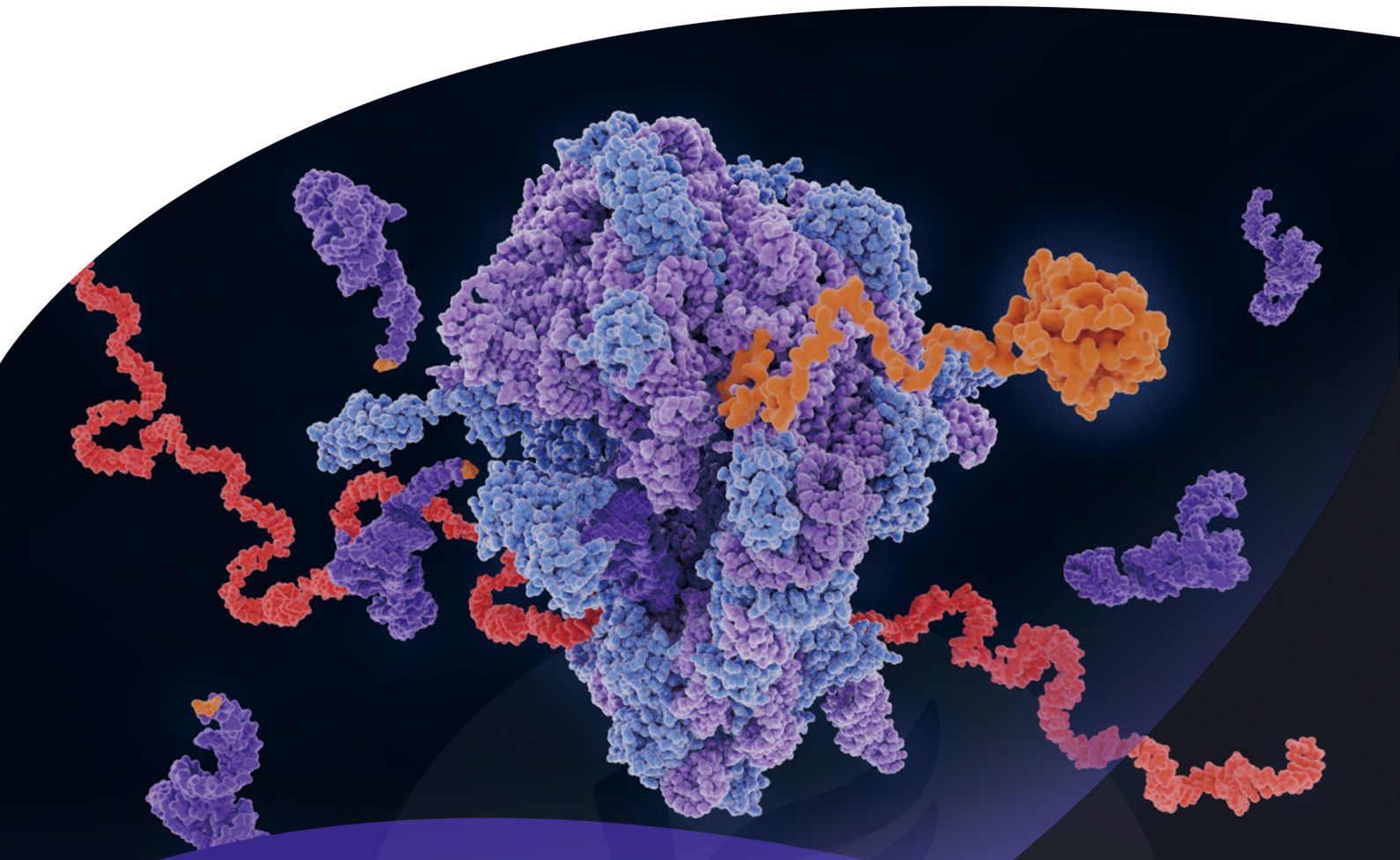
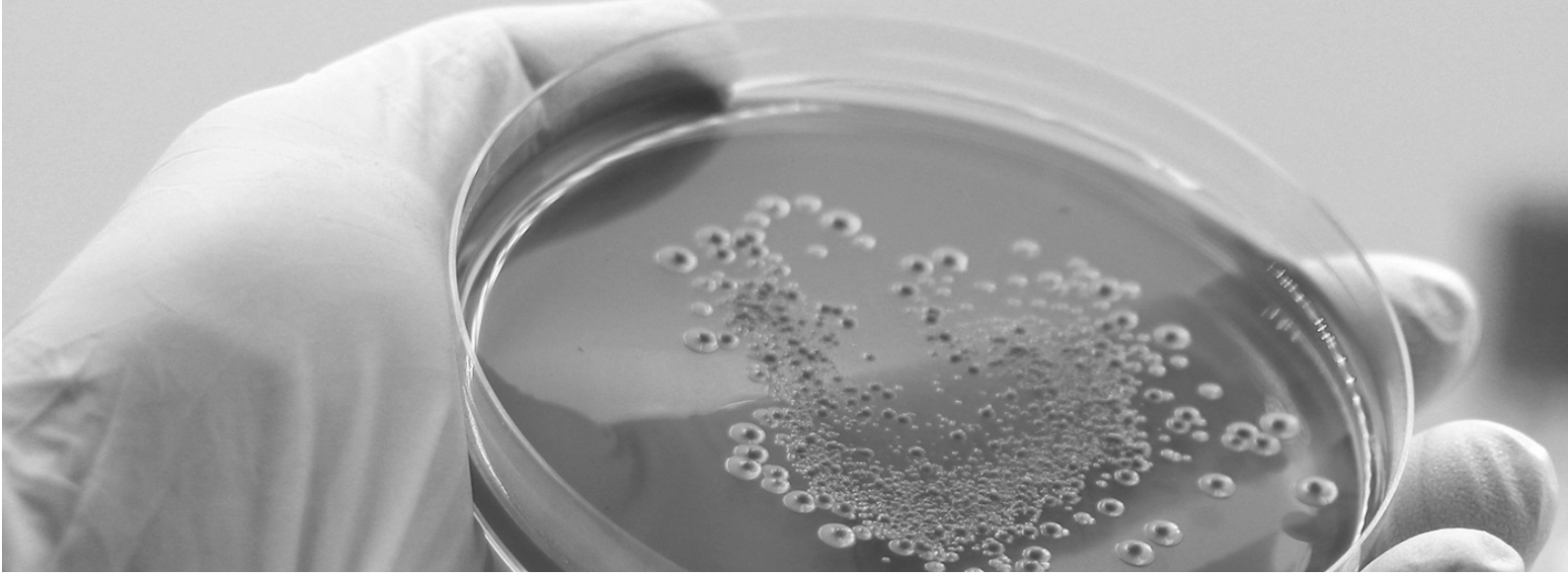
Recombinant Proteins CMO Services of Yaohai Bio-Pharma



YAOHAI BIO-PHARMA



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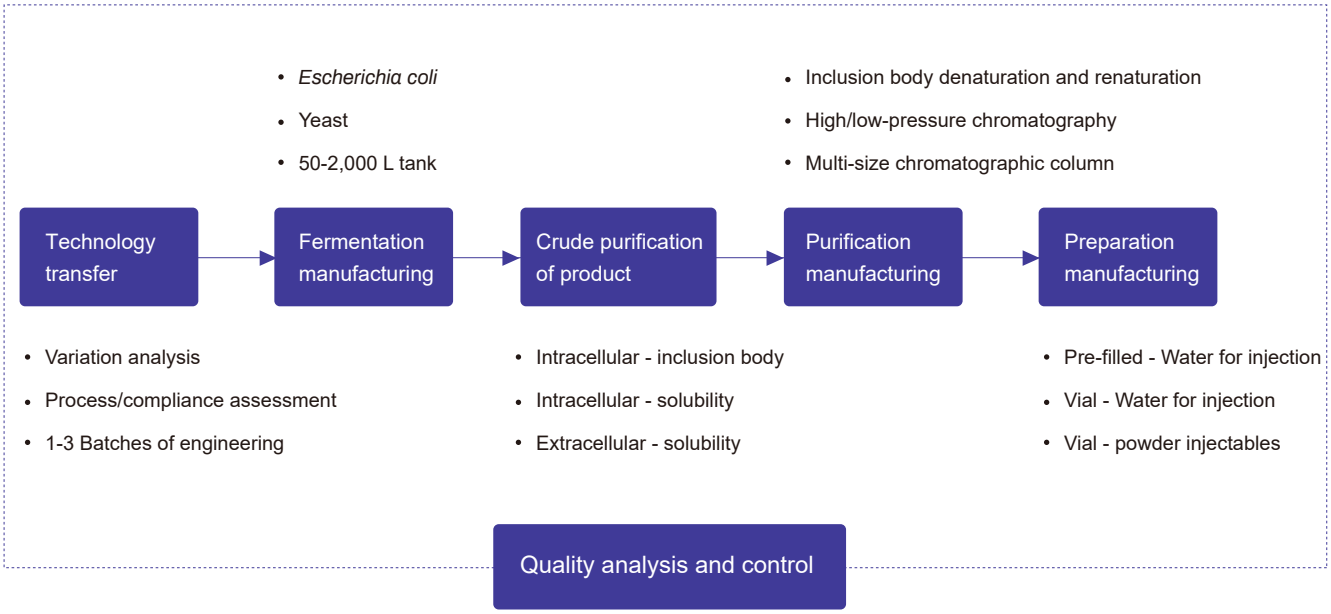


Yaohai Bio-Pharma is the preferred partner for customers in the field of microbial expression systems in China. We have extensive experience in recombinant proteins and recombinant plasmids manufacturing services. We focus on *Escherichia coli* and yeast expression systems, relying on GMP-level production workshops and the comprehensive quality management system. We take strict control of the process and the release criteria of raw materials, intermediates and final products of recombinant biologics enabling us to ensure batch-to-batch consistency. The BLA reporting strategy is adjusted according to the regulations of different countries to meet the requirements of our customers in both China and other regions.

Yaohai Bio-Pharma can provide GMP-level recombinant proteins manufacturing services, with multi-scale fermentation platforms of 50 L-100 L-200 L-500 L-1,000 L-2,000 L. The platform is equipped with multiple sizes of low/medium/high-pressure chromatography systems, and the automatically aseptic filling system for water for injection vials/lyophilization, pre-filled syringe/cartridge. Yaohai Bio-Pharma can provide the manufacturing services for IND application samples and phase I-III clinical samples, as well as the commercialization manufacturing service. Our service tenet is to help customers comprehensively accelerate the drug development process.

The CMO platform of Yaohai Bio-Pharma can serve the following recombinant protein products:

Recombination vaccines

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

Recombinant peptides

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.

Growth factors

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

Enzyme preparations

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

Nano-Antibodies

Nano-antibodies with different potencies (monovalency/bivalency/trivalency).

Collagens

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 peptides expressed with Escherichia coli/yeast

Service details

Service Name	Service Items	Service Details	Minimum Delivery Cycle (working days)	Deliverables
Technology transfer	Document transfer	Manufacturing process/analytical methods/quality specification	TBD	Process transfer report
	Assessment of technical and regulatory compliance	Variation analysis of man, machine, material, method and environment	1	
		Assessment of formulation and process	1	
		Assessment of analytical methods	3	
	Protocol transfer	Determination of overall transfer protocol	7	
	Process validation	Manufacturing of 1-3 batches of engineering	TBD Subject to customer' s process	
Recombinant proteins Fermentation manufacturing services	Confirmation before fermentation	Man, machine, material, method and environment	1	Intermediates
	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 proteins Crude purification manufacturing services	Confirmation before production	Man, machine, material, method and environment	1	
	Manufacturing preparation	Solution preparation	1-2	
	Crude purification of product	Collection and concentration of culture supernatant - optional	2	
		Collection and crushing of bacterial cells -optional	1	
		Collection and washing of inclusion body - optional	2	

Service Name	Service Items	Service Details	Minimum Manufacturing Cycle (working days)	Deliverables
Recombinant proteins Purification manufacturing services	Confirmation before purification	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	Confirmation before preparation production	Man, machine, material, method and environment	1	Vial-water for injection vials Vial-filing lyophilization Prefilled syringe-water for injection vials Cartridge-water for injection vials
	Pre-production preparation	Apparatus cleaning and sterilization	1-2	
	Preparation manufacturing	Filling of sterilized preparation	TBD Subject to customer' s process	
		Lyophilization-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 proteins

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 tests		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 proteins 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:
the mentioned “recombinant protein” generally refers to recombinant protein or recombinant polypeptides; 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.

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 water for injection vials (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.

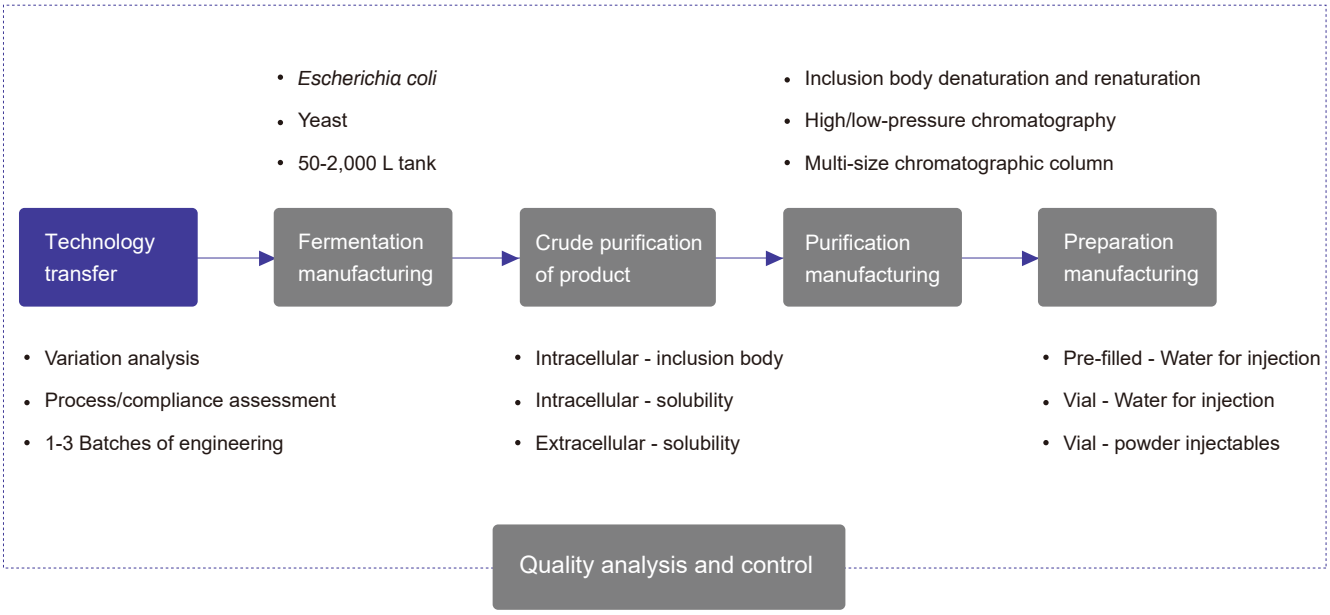
Extensive experience in technology transfer/scaling up

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.

Recombinant proteins technology transfer services



According to the ICH Q10 guidelines, the life cycle of a drug product is divided into four stages: drug development, technology transfer, commercial manufacturing, and product discontinuation. Technology transfer is an important part of the drug life cycle and is the key connecting link between drug R&D and commercial manufacturing. Technology transfer mainly includes manufacturing processes, intermediates control, quality specification of raw materials and excipients, 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. We will facilitate our customers to realize the transfer between commercial production and CDMO, CMO, CRO enterprises, to ensure the continuous and stable production of products.

Yaohai Bio-Pharma has established technology transfer management measures from small test process development, medium test production to GMP production stage (stock solution and preparation) in accordance with the Chinese Pharmacopoeia 2020 edition, ICH Q10, WHO, PDA TR65, ISPE and other technology transfer guidelines. We are always clear that the technology transfer process is based on the concept of Quality by Design (QbD). A comprehensive risk assessment has been conducted on the transfer process in terms of regulations and quality management, and the management of whole life cycles of drugs is strengthened, to ensure the success of technology transfer. And fully guarantee the safety, efficacy and quality control of drugs to our customers.

01 **Project launch**

- Team building
- Personnel training

02 **Document transfer**

- Manufacturing process
- Ouality 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	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

Extensive experience in process transfer

Fully assess the completeness and feasibility of process flow and the test methods, and provide customers with comprehensive process transfer solutions.

Compliance ensuring platform

Comprehensively assess the compliance of products and quality specification, 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
Fermenter	Culture volume, diameter-to-height ratio, mixing blade, maximum rotation speed	Aeration, rotation speed, dissolved oxygen	Tofflon
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 Yaohai Bio-Pharma Equipment column lists some equipment brands we have. Please consult our staff for more information.

During the process of technology transfer, Yaohai Bio-Pharma will perform parameter conversions for process transfer or scale up based on the inconsistent equipment models (e.g. fermenters, centrifuges and homogenizers). We will face differences in diameter-to-height ratio, stirring blade distribution and maximum speed of different brands of fermenters. Process validation and scale-up can be completed by controlling key parameters such as ventilation, rotational speed and dissolved oxygen. Different centrifugation equipment are available (benchtop type, floor type, drum type or disc stack type), and homogenizers with different capabilities are applicable for different volumes of samples. Therefore, during scaling-up process, the processes of some projects require conversion of centrifugation and homogenization equipment. The data that needs to be converted includes: centrifugation process parameters, including speed, feeding and residue discharging times (disc stack type), and the key homogenization parameters, including flow rate, pressure and times.

A conversion is required only for scale-up parameter under the condition that the Yaohai Bio-Pharma's equipment models are basically the same. During chromatography purification, with the column height and column efficiency being maintained within a controlled range, we maintain the retention time, loading capacity and elution conditions (linear flow rate) of the original process. Only the column volume and loading volume are required to be changed according to the actual scale. During filtration or ultrafiltration, we need to change the membrane area and control the flow rate according to the actual scale-ups.

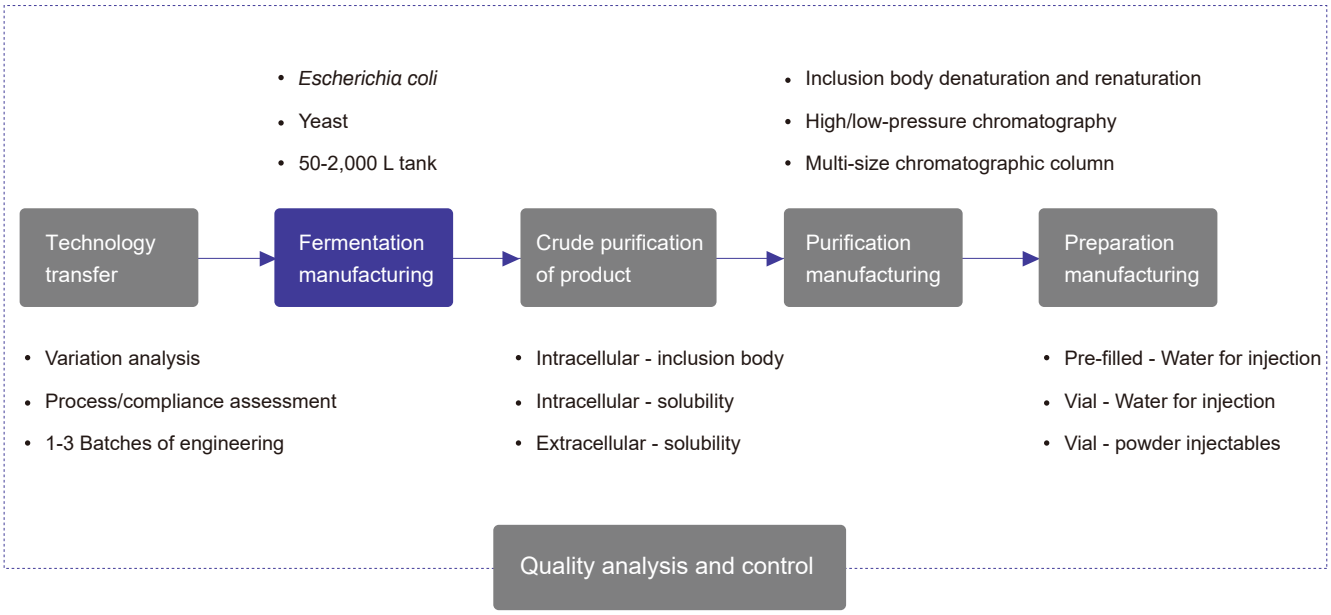
Based on the extensive CMO service experience of recombinant proteins/peptides/plasmids, Yaohai Bio-Pharma has accumulated experience in equipment-related process transfer of various brands, performances and models. We can quickly identify and adjust key equipment parameters to facilitate our customers to achieve fast delivery while maintaining the original quality of their products.

Yaohai Bio-Pharma TIP: 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
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Fermentation manufacturing services



Yaohai Bio-Pharma, in the field of microbial expression system, has extensive experience in the manufacturing services of recombinant proteins and recombinant plasmids. Relying on five independently operating GMP-level customized production lines and 50 L-140 L-200 L-500 L-1,000 L-2,000 L fermentation scales, we can meet different project needs of customers. Currently, Yaohai Bio-Pharma has served more than 100 customers at home and abroad, with extensive experience in industrial manufacturing of fermentation.

During the scale-up of fermentation process, process control parameters unrelated to scale are also kept consistent, including the culture and induction conditions (such as the basal medium, fed-batch medium, induction agent, temperature and pH), the process parameters of inoculation, feed supplement 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 the extensive experience in CMO services, Yaohai Bio-Pharma 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 proteins Fermentation manufacturing services	Confirmation before fermentation	Confirmation of man, machine, material, method and environment	1	Intermediates
	Preparation before fermentation manufacturing	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	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, and comply with all specifications of the latest GMP requirements.

Multi-size fermentation system

There are five production lines for stock solution, which are built in accordance with international GMP requirements, and can provide mixing and ventilating fermenters with sizes of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, to support the production needs at different development stages.

Diversified fermentation platform

To meet the needs of customer projects for high-density fermentation processes, customized fed-batch and induction processes of Escherichia coli and yeast with or without antibodies.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements.

Experience sharing of fermentation process scale-up

The key parameters of the 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 extensive experience in CMO production services, Yaohai Bio-Pharma has summarized the issues frequently occurred during fermentation process transfer or scale-up process and the scale-up strategies:

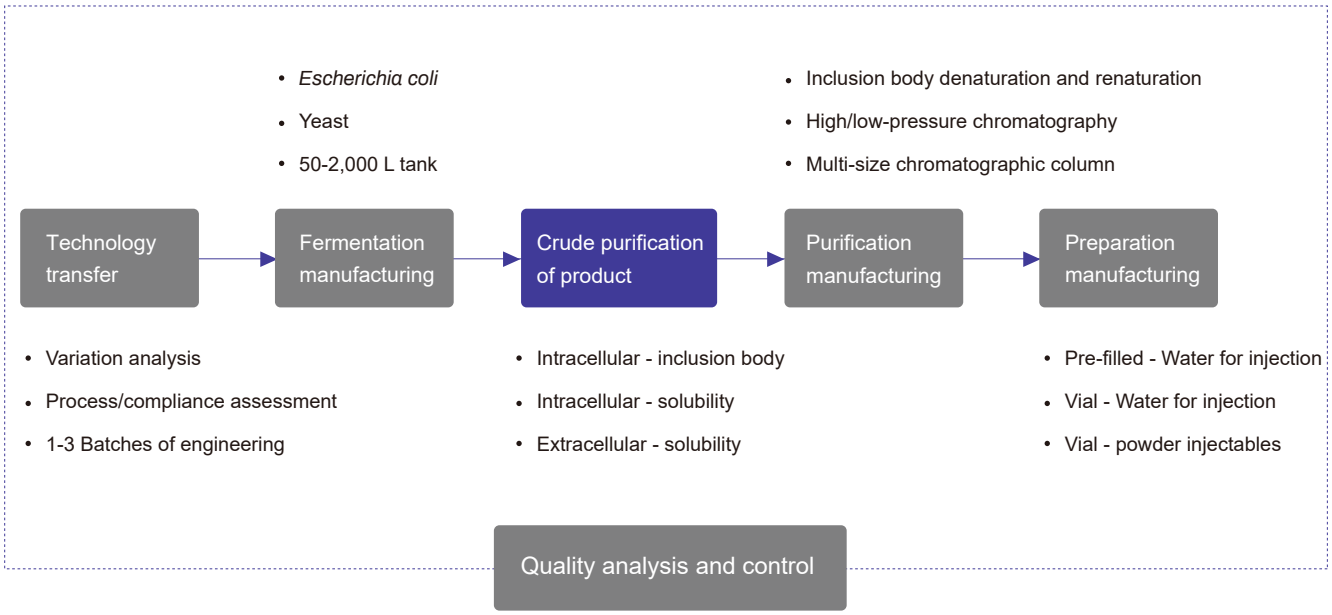
Parameter types	Related parameters	Frequently asked questions	Prevention or solutions
Questions related to culture condition	Temperature and pH	[Volume-independent parameters, consistent]	The temperature, pH sensor, pump and other equipment are calibrated and tested under GMP standards.
	Feeding strategy		
	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 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 amount of the fermentation process during process transfer or scale-up?	The purpose of aeration is to provide oxygen for bacterial cells, 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 validated 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 amount, 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 amount.
	$OD_{600\ nm}$	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, 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
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Crude purification manufacturing services



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.

In the field of microbial expression systems, Yaohai Bio-Pharma has extensive experience in the manufacturing services of recombinant proteins and recombinant plasmids. We rely on five independently operating GMP-level customized production lines, which are equipped with centrifuges and homogenization equipment of different processing batches. There are fermentation tanks of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, which can meet the needs of different customers. Based on the extensive experience in CMO services, corresponding parameter conversions for centrifuges and homogenizers of different scales/performance can be performed. The key parameters can be controlled, which can successfully realize the scale-up production of crude purification process and the effective removal of some impurities.

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

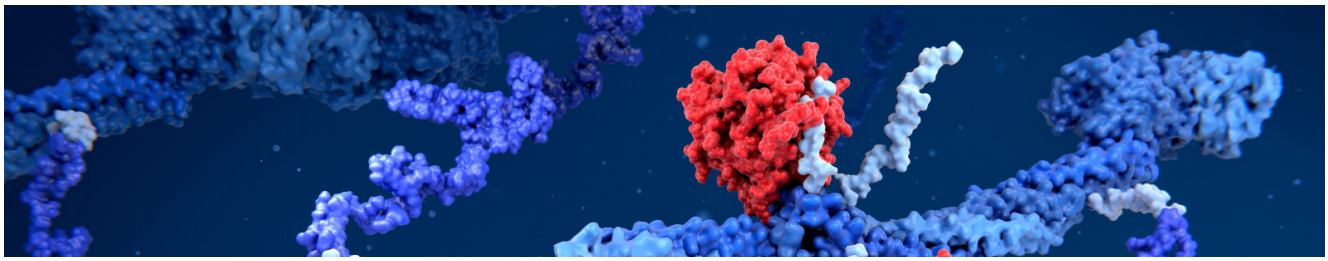
04 Inclusion body collection

- 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 proteins Crude purification manufacturing services	Confirmation before production	Confirmation of man, machine, material, method and environment	1	Intermediates
	Preparation before production	Buffer solution preparation	1-2	
	Extracellular soluble form-optional	Supernatant collection by centrifugation	2	
		Concentration and solution replacement		
	Intracellular soluble form-optional	Collection of bacteria cells by centrifugation	2	
		High pressure homogenizing and crushing		
		Centrifugal removing of bacterial debris		
	Intracellular inclusion bodies-optional	Collection of bacteria cells by centrifugation	3	
		High pressure homogenizing and crushing		
		Collection of inclusion bodies by centrifugation		
		Inclusion body washing and subpackage		
Line clearance	Workshop line clearance	Equipment cleaning, 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, and comply with all specifications of the latest GMP requirements.

Multi-scale crude purification equipment

There are five GMP-level production lines for stock solution, equipped with bench top type/drum type/disc stack type 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 releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements

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.

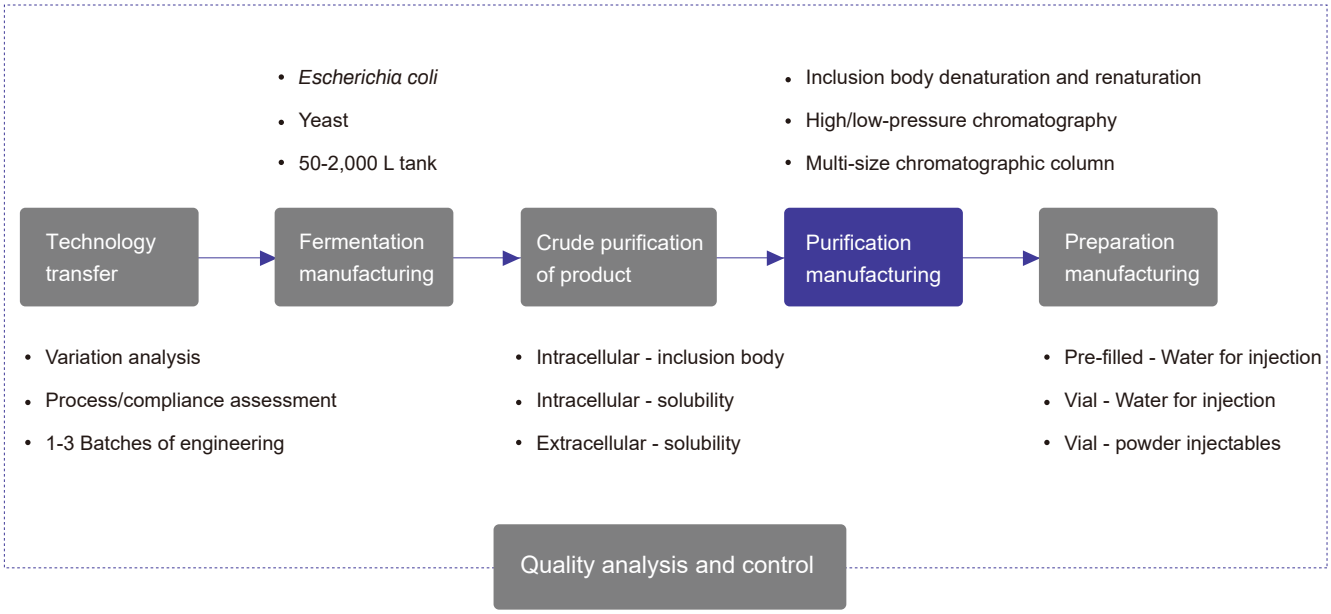
Based on our extensive experience in crude purification service of products, Yaohai Bio-Pharma has summarized the questions frequently occurred during the transfer of centrifugation and high pressure homogenization process and the solutions:

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
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Purification manufacturing services



In the field of microbial expression systems, Yaohai Bio-Pharma has extensive experience in manufacturing services of recombinant proteins. The purification workshop is equipped with different sizes of automatic or manual membrane filtration systems and low/medium/high-pressure chromatography systems. The following purification services are available: filtration and 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, and ultrafiltration solution replacement. Currently, Yaohai Bio-Pharma has served more than 100 customers at home and abroad and has rich experience in industrial manufacturing of purification.

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 proteins Purification manufacturing services	Confirmation before purification	Confirmation of man, machine, material, method and environment	1	Recombinant proteins-stock solution
	Preparation before purification	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	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		
		Enzyme digestion, modification, and coupling-optional		
		Concentration and solution replacement		
		Filtering 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, and comply with all specifications of the latest GMP requirements.

Multi-size fermentation system

There are five production lines for stock solution, which are built in accordance with international GMP requirements, and can provide mixing and ventilating fermenters with sizes of 50 L-140 L-200 L-500 L-1,000 L-2,000 L, to support the production needs at different development stages.

Standard GMP-level explosion-proof worksho

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, which satisfies the needs of solution dispensing in special process, such as reversed-phase chromatography.

Compliant testing and releasing specification

The brand and batch number of materials (raw materials and excipients) are verified, and the key materials are tested for releasing to ensure consistency and effectiveness of the materials.

Single project operation system

Only one project is allowed to be operated in each workshop during each time period to effectively prevent contamination and mix-ups, and the subsequent project shall be carried out only after the line clearance passes the requirements.

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

Experience sharing of purification process scale-up

Filtration and clarification are essential for the manufacturing of chromatography purification. Clarification is designed to further remove particulate substances to avoid posing negative impacts on the purification process in the downstream, which is usually completed using hollow fiber or membrane cassette. Key parameters in the process transfer or scale-up include the processing 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, Yaohai Bio-Pharma has summarized the questions frequently occurred during the transfer of purification process and the scale-up strategies:

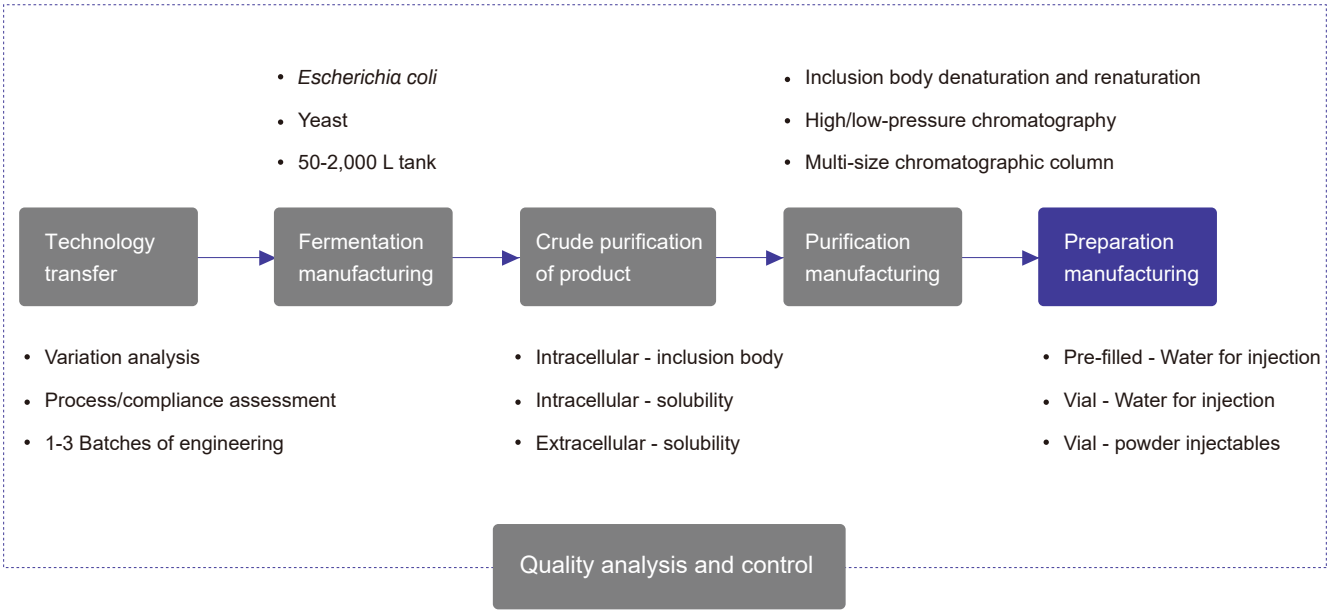
Purification process	Frequently asked questions	Process scale-up strategies
Filtration and clarification	What if there is no clarification process, or this process step is omitted in the small tests or medium tests?	*Suggestion*: The samples should be clarified during the process scale-up to remove the 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 aperture, 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 a conforming stainless steel tank.

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

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Preparation manufacturing services



Yaohai Bio-Pharma relies on the GMP-level high-tech automatic production lines for sterile biopharmaceutical preparations, including multiple processes of vial washing, drying, sterilizing, aseptic filling, lyophilization, capping, and etc. We can provide services for different dosage forms of preparations, such as Water for injection vials, vials filling lyophilization, pre-filled water for injection vials (pre-filled syringe/cartridge).

The sterile preparation production lines of Yaohai Bio-Pharma conform to the manufacturing requirements for sterile preparations of US FDA, EU EMA, China NMPA and Australia TGA. We can provide the services of formulation preparation and aseptic filling of drugs and placebos, to meet the needs of different customers for IND application, phase I-III clinical research, and MAH commercialization.

Yields / Dosage form	Water for injection vials 1 mL-25 mL	Vial powder injectables 1 mL-25 mL	Pre-filled syringe/cartridge water for injection vials 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	10 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 proteins Preparation manufacturing services	Confirmation before preparation production	Confirmation of man, machine, material, method and environment	1	Vial-water for injection vials Vial-filing lyophilization Prefilled syringe-water for injection vials Cartridge-water for injection vials
	Preparation before production	Receipt of documents and materials		
		Reconfirmation of conditions before production in GMP workshop		
	Apparatus preparation	Apparatus cleaning and sterilization	1	
	Preparation manufacturing	Vial sorting and vial washing	1	
		Formulation preparation-optional	1	
		Sample sterilization and filtration		
		Filling and stoppering (normal/nitrogen filling/vacuum)	1	
		Lyophilization-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 and sterilization and environmental disinfection	-	

Note: TBD: to be determined (subject to customer's process and batch size);
The current preparation workshop can provide the production of water for injection vials/filling lyophilization, pre-filled water for injection vials (pre-filled syringe and cartridge), and communication on other dosage forms are also welcomed.

Service features

Mature GMP training system

The workshop staff and QA/QC personnel have been strictly trained and instructed under GMP, and comply with all specifications of the latest GMP requirements.

Diversified preparation types

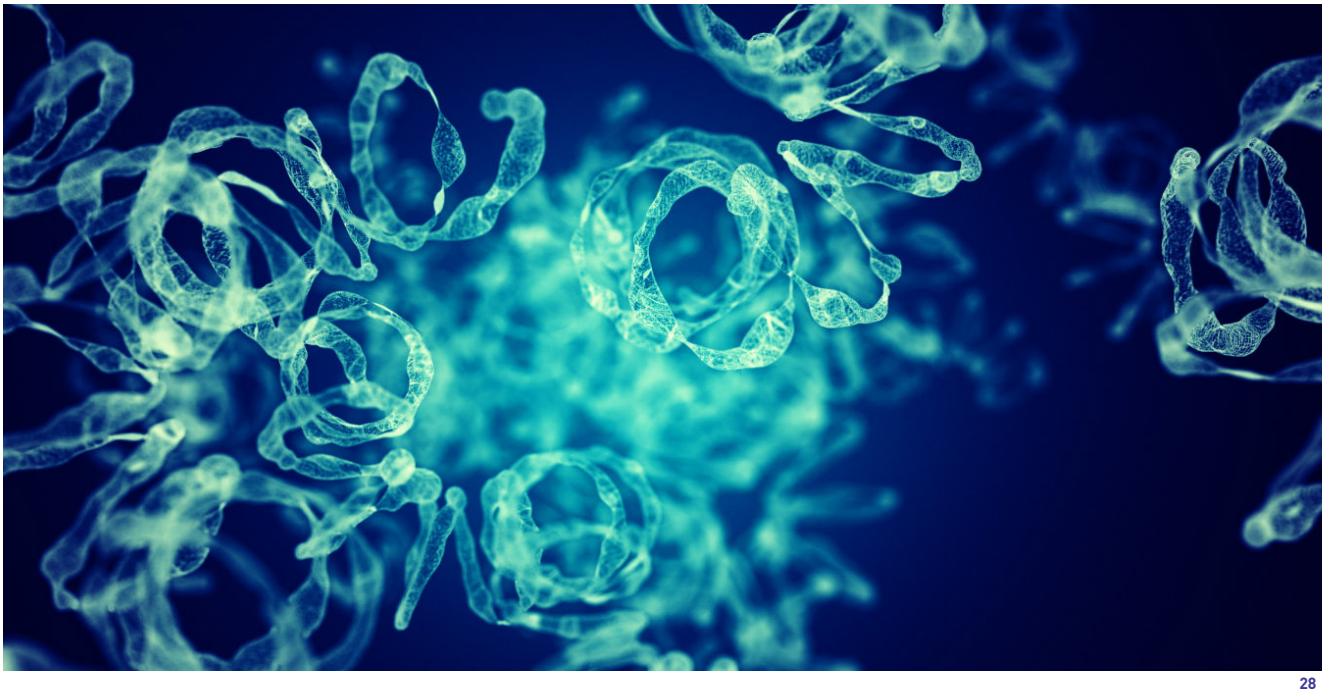
GMP-compliant automated sterile preparation production lines can serve the following products: 1-25 mL vial water injectables/filling lyophilization, 1-3 mL pre-filled syringe/cartridge water injectables.

Aseptic preparation filling production line

Conforming to aseptic preparation manufacturing requirements of US FDA, EU EMA, China NMPA and Australia TGA. O-rabs system (Open Restricted Access Barrier System) are used to protect the exposure areas of products (and the packaging materials), providing grade A environmental protection under grade B background.

Extensive project experience

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



Experience sharing of aseptic preparation process scale-up

With extensive experience in preparation filling service, Yaohai Bio-Pharma 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.

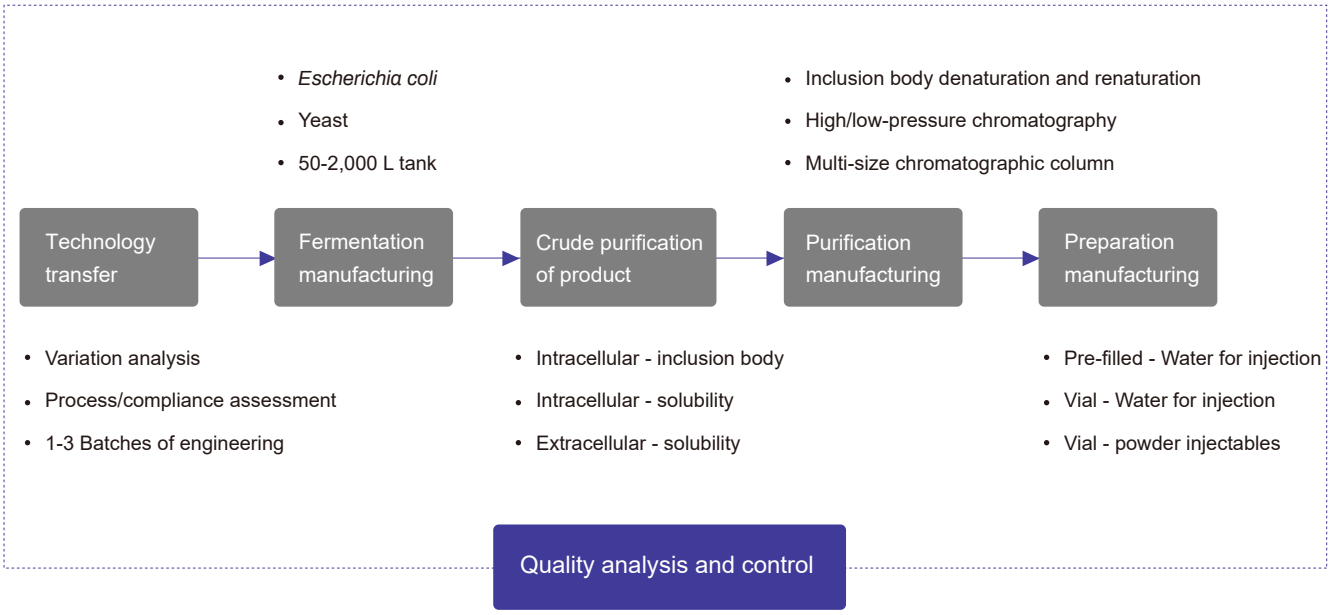
Purification process	Frequently asked questions	Yaohai BioPharma' s experience
Lyophilization	Why does the freeze-dried powder appear the phenomenon of wall climbing traces of drug solutions?	The phenomenon of wall climbing traces is related to the characteristics of the drug solution (active ingredient of drug 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 vials, may also lead to the wall climbing traces of drug solutions.
	How to improve if there is the phenomenon of wall climbing traces of lyophilized 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 lyophilization 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. Such phenomenon may appear in only a few varieties and surfactant is contained in excipients.

Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

Quality analysis and control services



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, tests of products. Quality control involves assessment of known/potential products and process-related substances by using standard substances and validated methods, and analysis of test items of product appearance identification, activity, purity and impurities.

Yaohai Bio-Pharm has a comprehensive quality analysis and control system. Our team members have thoroughly proficient in pharmacopoeia and other regulatory specifications. They have own extensive experience in quality testing and analysis. We are able to implement sample tests in conformity with the specifications, guarantee 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 and excipients - full tests		11
	Packaging materials		60
Recombinant proteins 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 proteins Quality analysis and control	Antibiotic residue	ELISA, culture method	5
	Microbial limit test	Plate method, membrane filtration method	10
	Sterility 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:
The mentioned "recombinant proteins" generally refers to recombinant proteins or recombinant peptides; 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 proteins stock solution + preparation, the average delivery cycle of Yaohai Bio-Pharma 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

The QA/QC personnel have been strictly trained and instructed under GMP comply with all specifications of the latest GMP requirements.

Compliant QC testing process

Being able to reasonably assess the compliance of analytical methods and quality release specifications, and can quickly complete the transfer and validation of the analytical methods.

Whole-process quality control

The raw materials and excipients, package materials, intermediates, stock solution and preparations of recombinant proteins are tested for releasing, with the quality specification of materials and samples strictly controlled.

Complete quality analysis platform

Based on our extensive experience in CMO services, the quality control team of Yaohai Bio-Pharma 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.

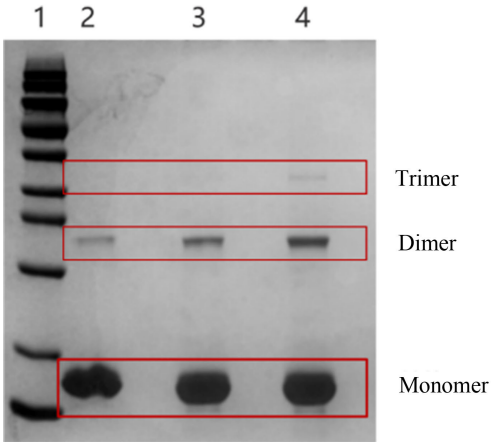
Quality analysis case sharing

Improper pretreatment method of the test samples can pose certain impacts on the quality test results.

In a purity test of the lyophilized powder of recombinant proteins, different volumes of re-suspension solution were used by Yaohai Bio-Pharma for re-dissolution to obtain protein samples at different concentrations: 1 mg/mL, 5 mg/mL and 10 mg/mL, and the 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 monomeric form. 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]



The quality control team of Yaohai Bio-Pharma has established a highly applicable, robust and reliable analysis platform, by which the compliance assessment, method transfer and validation of quality test methods can be accomplished, to match against the product quality requirements in a high-standard way.

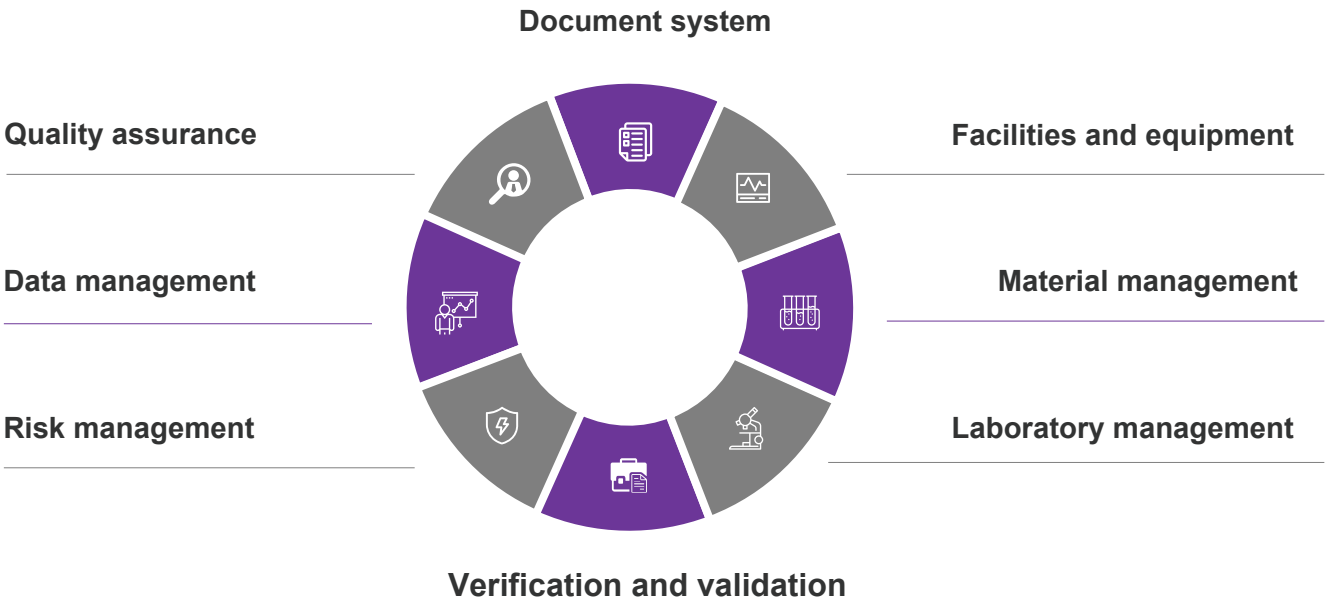
Other Services

- Technology transfer
- Fermentation manufacturing services
- Crude purification manufacturing services
- Purification manufacturing services
- Recombinant protein preparation manufacturing service
- Quality analysis and control service

GMP quality assurance 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 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.

The bio-quality system management personnel in Yaohai Bio-Pharma have GMP certification experience, and the executive team has GMP work experience. Our team members are proficient in studying, interpreting and translating global regulations. We have developed a compliant quality management system by combining different life cycle stages of drugs. We also manage and control the whole process of man-machine-material-method-environment in the production stage.



Document system

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

Quality assurance

• System management: Document/record, training, change/deviation/CA PA/complaints, self-test, material/supplier manage
ment
• 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

Verification and validation

• Verification of plant and facilities
• Equipment verification
• Computerized system validation
• Process validation
• Metrology management
• Cleaning verification
• Aseptic process simulation
• Validity period verification, etc.

Laboratory management

• Management of samples/references, reagents and consumables
• Verification and validation of analytical methods, management of entrusted testing
• Data, record and report management, quality information management

Material management

• 1,400 m2 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

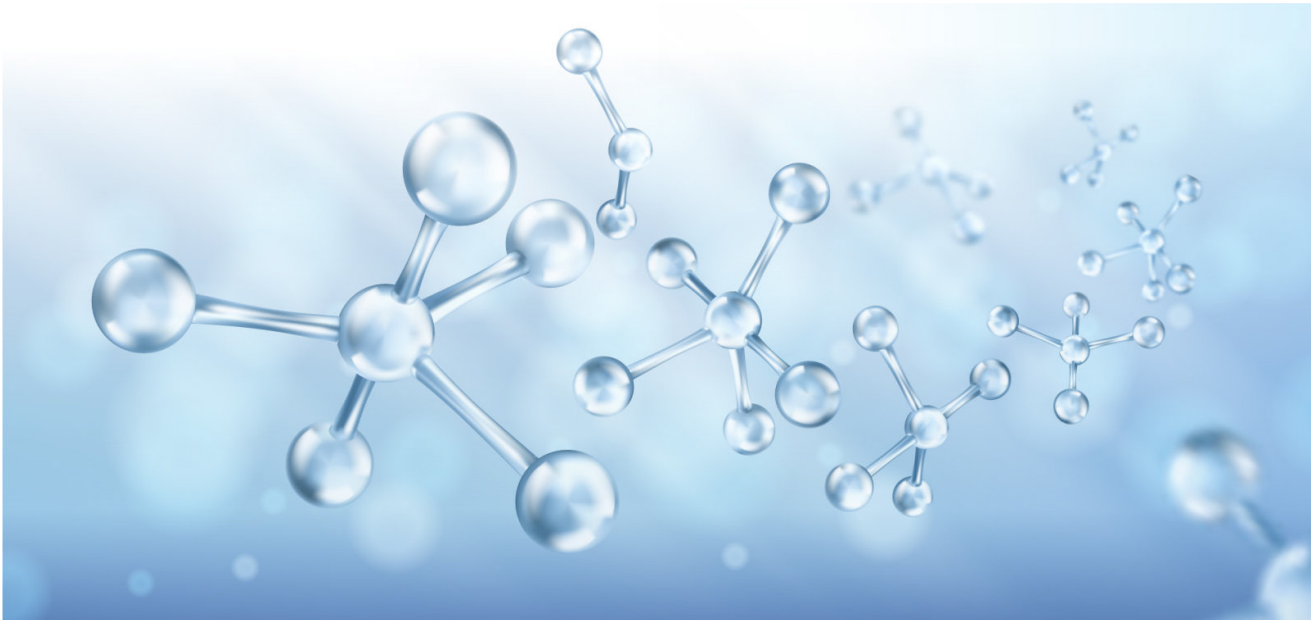
Facilities and equipment

• 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, on-line monitoring, validation and measurement

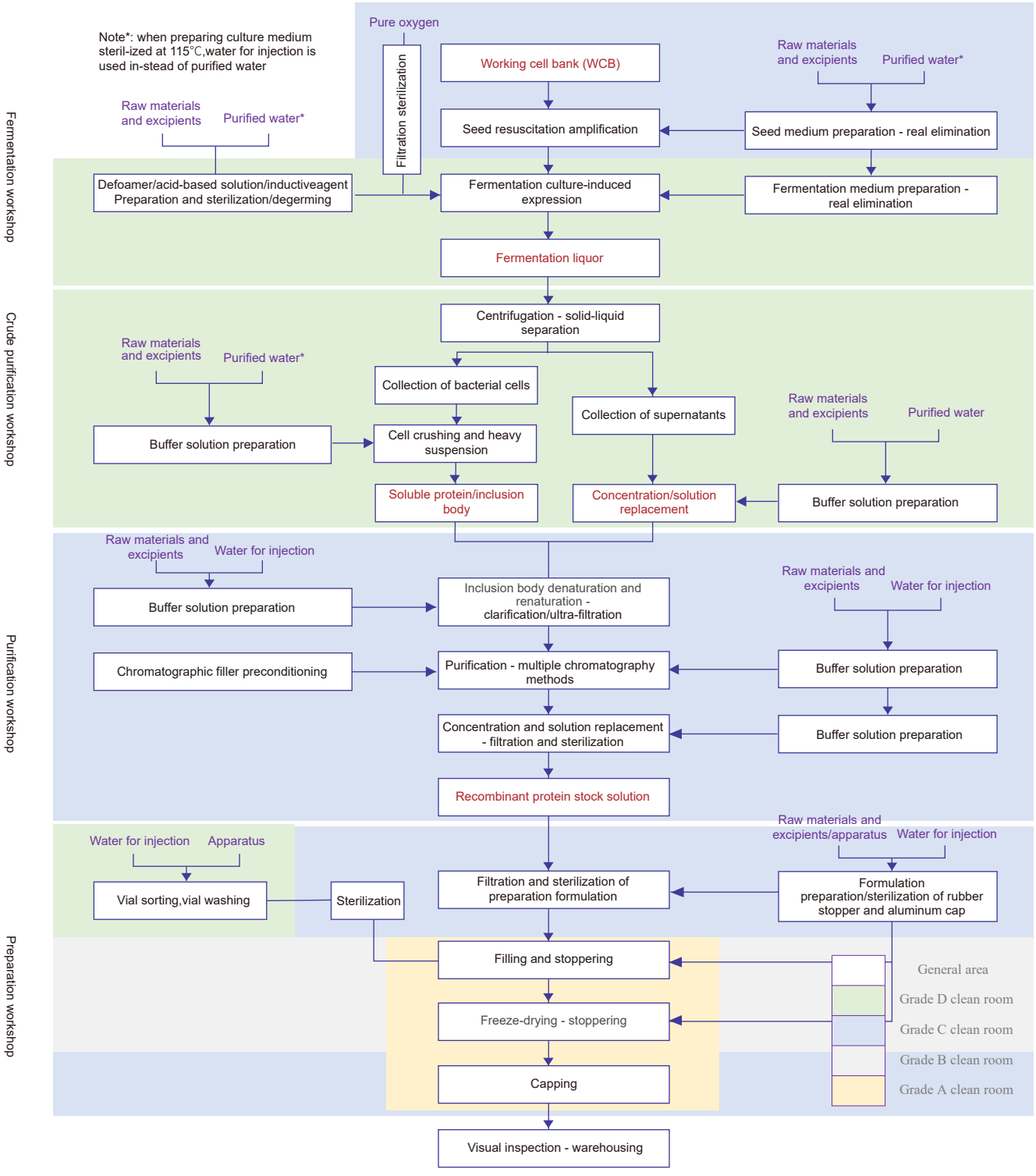
Management and control of clean room in GMP workshop

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



Functional area of GMP workshop



Presentation of GMP workshop and equipment

Solution dispensing system

Fermentation system

Disc stack type centrifuge

Cartridge centrifuge

Homogenizer

Hollow fiber system

Low-pressure chromatography system

High-pressure chromatography system

Aseptic filling system

Capillary electrophoresis instrument

Gas Chromatograph

Liquid chromatograph

SERVE
WITH HEART &
CREATE
THE FUTURE TOGETHER

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