Yaohabio
耀海生物

FROM CONCEPT
TO VACCINES
ONE-STOP CRDMO

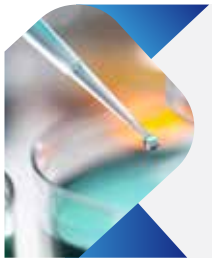
YAOHAI BIO-PHARMA VACCINE ONE-STOP SERVICE PLATFORM

CONTENTS

ABOUT YAOHAI BIO-PHARMA

Yaohai Bio-Pharma is China's first and largest biologics CRDMO (Contract Research, Development and Manufacturing Organization) specializing in microbial expression systems. It was established in China Medical City (CMC) in August 2010. The company offers customized end-to-end solutions from DNA design and synthesis, microbial strain engineering and construction to drug substance manufacturing in GMP or non-GMP level and fill & finish products across diversified modalities, such as recombinant proteins, peptides, and polypeptides, enzymes, single-domain antibodies (sdAbs), plasmid DNA and mRNA, glyco-polymers, virus-like particles (VLPs). Yaohai Bio-Pharma is a leading player in the industry, capable of meeting the clinical and commercial requirements of global customers in the fields of biological drugs, biosimilars, vaccines and diagnostics for both human and veterinary use.

Adhering to the service concept of serving with heart creates the future together. We persevere to invigorate global new drug development with the mission of establishing international standards, boosting new drug development processes, and achieving a healthy life.



300+ Genetically Modified Bacteria (GMB)

We have accumulated over ten years of experience in successfully completing the development of more than 300 GMB projects for our customers. Our target products encompass plasmid DNA, recombinant peptides, recombinant enzymes, single-domain antibodies and other types of proteins.

0.5~15 g/L

The strain produces the target product at a yield of 0.4~15 g/L.
The yield of plasmid DNA > 0.5g/L
The yield of soluble protein ranges from 0.5 to 15 g/L.
The yield of inclusion body protein ranges from 2 to 10 g/L

Overview of Vaccine CRDMO Services

Vaccine Applications

Vaccines prompt the body to create a strong immune response against harmful substances either inside or outside the body. Furthermore, they can create a long-lasting immune memory. Based on the target recipients, vaccines can be divided into human and veterinary vaccines.

Vaccines can be classified into prophylactic vaccines and therapeutic vaccines based on their intended use. Prophylactic vaccines stimulate the body to produce neutralizing antibody (Nab), which can kill viruses or pathogens. On the other hand, therapeutic vaccines induce the production of antibodies to target tumor cells or block specific metabolic pathways.

Human Veterinary

Human Prophylactic Vaccines

- Viral Infections
- Bacterial Infections
- Parasitic Infections
- Toxin-induced Poisoning

etc.

Human Therapeutic Vaccines

- Tumors
- Cardiovascular Diseases
- Infectious Diseases
- Autoimmune Diseases
- Neurological Diseases

etc.

Veterinary Prophylactic Vaccines

- Viral Infections
- Bacterial Infections
- Parasitic Infections
- Toxin-induced Poisoning
- Tumors

etc.

Veterinary Therapeutic Vaccines

- Tumors

etc.

Source: World Vaccine Congress

Vaccine CRDMO Services of Yaohai Bio-Pharma

Vaccines are made using several processes. They may contain live- attenuated organisms, inactivated organisms, inactivated toxins, antigens or antigenic epitopes, like subunit and conjugate vaccines, DNA/mRNA, virus vector or microbial vector encoding the target antigens or antigenic epitopes.

Yaohai Bio-Pharma offers a range of vaccine services using microbial fermentation systems, as follows:

Technical Route	Business	Deliverables (Intermediate/Drug Substance/Drug Product)	Services
mRNA Vaccines	Yes	DS or DP: mRNA or LNP-mRNA Our partner, NanoStar, licensed their own LNP patents to us.	Vaccines CRDMO services, including intermediates, drug substances (DS) or drug products (DP) 【GMP grade】 • Research samples preparation, such as mRNA, DNA or proteins; • Microbial cell banking; • Process development, such as fermentation, purification and formulation; • Analysis method development; • GMP manufacture of mRNA, DNA, proteins, live bacteria (BSL-2 Laboratory) • Quality control of the products • Drug registration
DNA Vaccines	Yes	DS or DP: Plasmid DNA	
Viral Vector Vaccines	Yes	Intermediate: Plasmid DNA	
Subunit Vaccines	Yes	DS or DP (adjuvant): recombinant antigens-based vaccines	
Protein/Peptide-based Therapeutic Vaccines	Yes	e.g. Prophylactic vaccines for HPV, RSV, or therapeutic vaccines for cancer, hypertension.	
Conjugate Vaccines	Yes	DS or DP: Conjugate vaccines e.g. Pneumococcal, meningococcal vaccines. Intermediate: Carrier proteins e.g. VLP, CRM197, tetanus toxin, etc.	
Polysaccharide Vaccines	Yes	DS or DP (adjuvant): Polysaccharide vaccine e.g. Typhim Vi vaccine, pneumococcal vaccine.	
Toxoid Vaccines	Yes	DS or DP: Inactivated toxoid e.g. Diphtheria, tetanus and pertussis toxin, etc.	
Live-attenuated Vaccines (Microbial)	Yes	DS or DP: Live-attenuated vaccines e.g. BCG live, cholera vaccine live	
Microbial-vector Vaccines	Yes	DS or DP: Live microbial products	
Live-attenuated Vaccines (Virus)	No	Not applicable	Not applicable
Inactivated Vaccines	No	Not applicable	Not applicable

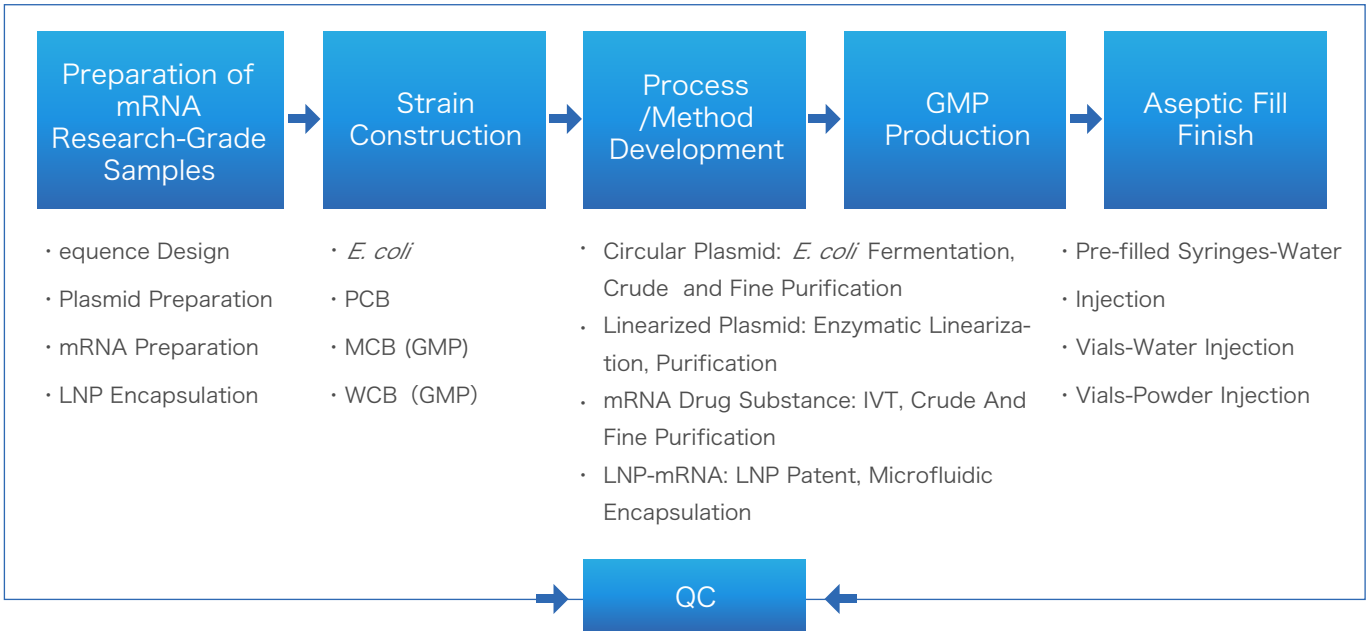
mRNA Vaccine CRDMO Services

With the widespread administration of mRNA COVID-19 vaccines in large populations, the safety of mRNA vaccines has been validated. mRNA possesses the ability to express any protein, offering potential solutions to various unmet clinical needs.

Yaohai Bio-Pharma provides a comprehensive solution for mRNA development and GMP production, backed by a robust research platform and a compliant GMP system. Our services are tailored to meet the unique requirements of our clients, offering them high-quality mRNA drug substances, LNP-mRNA finished products in different specifications, detailed development and production reports, and testing reports.

We have obtained authorization for LNP patent technology from our partner, NanoStar Pharmaceuticals, ensuring the avoidance of potential patent disputes in the future.

mRNA/LNP One-Stop Solution



Product Grade	Deliverable Products	Deliverable Specifications	Sample Applications
non-GMP	Drug Substance of mRNA	0.1~10 mg (mRNA)	Preclinical Research Such As Cell Transfection
	Drug Product of LNP-mRNA		
GMP, Sterility	Drug Substance of mRNA	10 mg~70 mg	IND/CTA Clinical Trial Samples Commercialized Products
	Drug Product of LNP-mRNA	5000 (Pre-fill and finish/ Vials)	

mRNA CRDMO service, covering the entire life cycle of mRNA

Discovery, Research → IND/CTA → Phase 1/2 → Phase 3 → BLA/MAA → Commercial

Early Research

Preparation of mRNA Research-Grade Samples
- Sequence Design & Optimization
- Plasmid Construction
- mRNA synthesis in vitro
- Formulation Studies of Drug Product
- Quality control
- In Vitro Activity Studies

Strain Engineering Development
- Host strain screening Establishment and Validation of a Tier 3
- Microbial Strain Bank

Process Development and Optimization

Process Development and Optimization
- Plasmid: Fermentation, Purification , Linear process development and optimization
- mRNA: IVT, capping, development and optimization of Purification
- Drug Product : LNP Formulation Development, Process Development and optimization of LNP encapsulation ;

Process Characterization and Process Validation, Process Performance Qualification (PPQ)

Analytical Method Development and Validation
- Method Development and Validation (Intermediate, Drug Substance, Drug Product, Impurities)
- Control of Critical Quality Attributes: Identification, Integrity, Purity
- Pre-stability Studies, Drug Substance and Drug Product Stability Studies

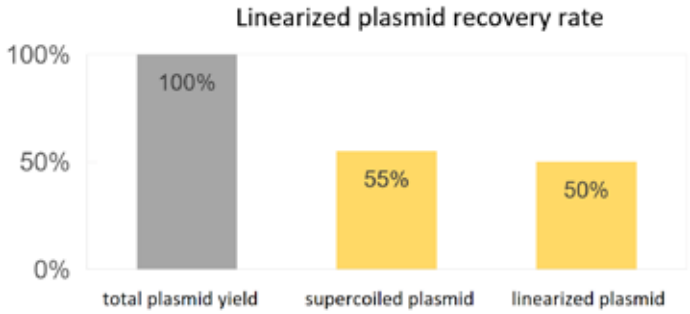
Technology Transfer and GMP production
- Technology Transfer
- Raw Material Testing and Release
- GMP Drug Substance, Aseptic Formulation Registered Batch Production
- GMP Drug Substance, Aseptic Formulation Clinical Sample Production
- GMP Drug Substance, Aseptic Formulation Commercial Product Production
- QA and QC

- Preparation of CTD Format Regulatory Submissions
- Global Registration Services

Platform Features

Plasmid DNA Platform

- Multiple 7L fermentation systems, animal-free throughout the process
- Clear traceability of plasmids and host bacteria, with no declaration obstacles
- Plasmid yield with polyA exceeding 500 mg/L PolyA loss rate less than 5 bp
- Supercoiled plasmid proportion greater than 90%, with a recovery rate of over 55%
- Linearization efficiency exceeding 99%, with a linearized plasmid recovery rate of 90%

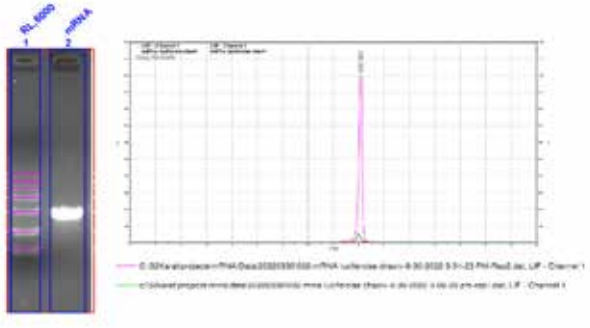


Drug Substance Platform of mRNA

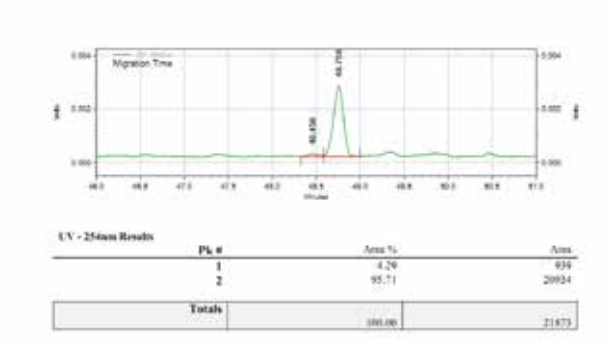
- Multiple 1L Reactors (GMP)
- mRNA integrity exceeding 98%
- 1: High transcription efficiency ratio of 120, allowing for scalable IVT process

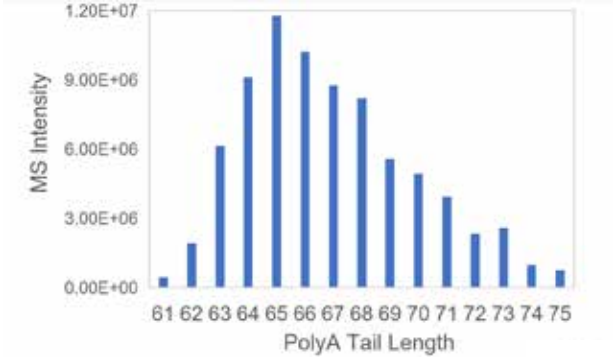
Reaction Specification	Transcription Efficiency
1ml	1:160
10 ml	1:150
50 ml	1:125
200 ml	1:120

Stable capping process with a capping rate of over 95%



Transcription templates with A-tails (two-step), ensuring uniform distribution of polyA tails.



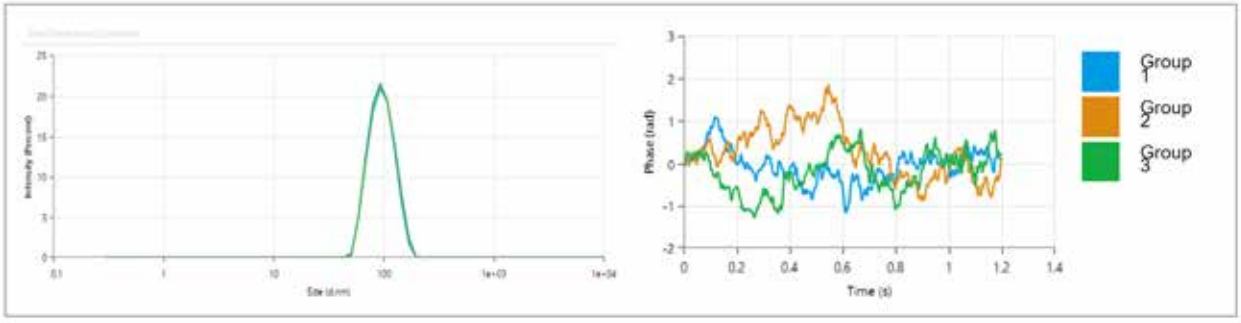


LNP Encapsulation Platform

- LNP patent technology authorized by our partners to ensure avoidance of patent disputes for our customers.

- Employing a highly versatile microfluidic encapsulation process, achieving an encapsulation efficiency of over 95%.
- Controlling LNP particle size within the range of 80-100 nm, with a low polydispersity index (PDI) of 0.05, indicating a uniform distribution of particle sizes.
- LNP particles exhibit a weak charge, with a Zeta potential of approximately -2.18 mV.

Testing Item	Testing Method	Testing Result
Encapsulation Efficiency	Ribogreen	92.7%
Particle Size	Malvern	92.07 nm
PDI	Malvern	0.05
Zeta	Malvern	-2.18 mV

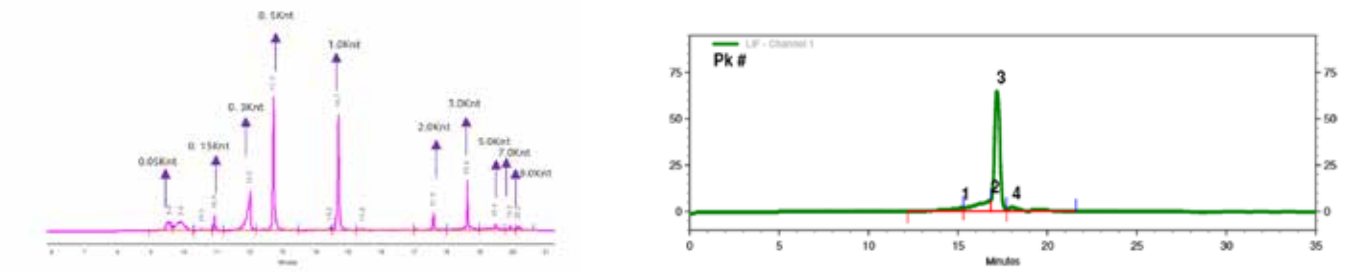


Method Development Platform

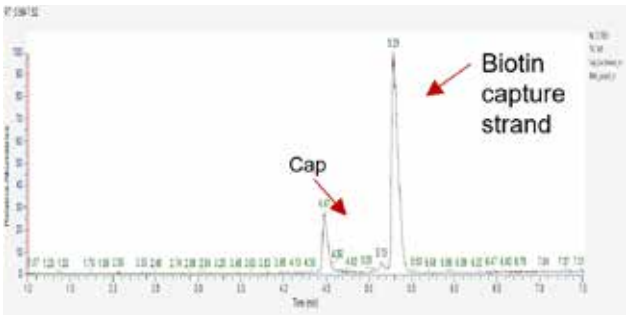
We offer a comprehensive method development platform for analyzing a wide range of targets, including circular and linearized plasmids, mRNA raw materials, and finished LNP-mRNA products. Our analysis covers a variety of parameters, such as integrity, purity, capping efficiency, polyA distribution, encapsulation efficiency, particle size, LNP components, and various process residuals (HCP, HCD, HCR, dsRNA, antibiotics, DNase I, T7 RNA polymerase, vaccinia capping enzyme, 2-O methyltransferase, etc.).

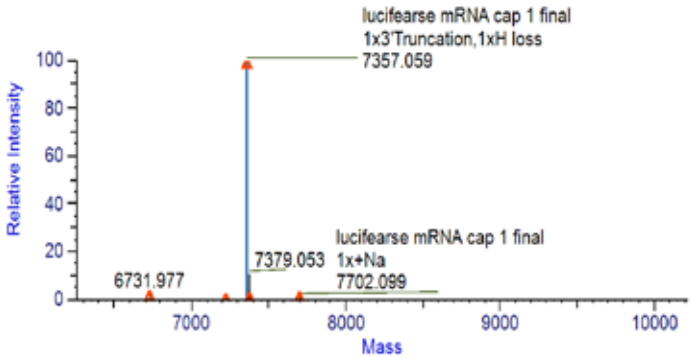
Partial methods are demonstrated as follows:

Detection of mRNA Integrity (Capillary Electrophoresis)



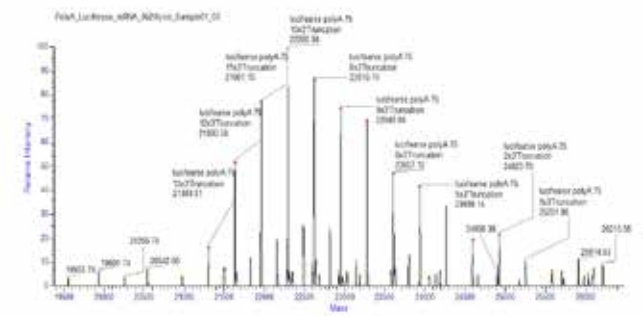
Detection of mRNA Capping Efficiency (LC-MS)

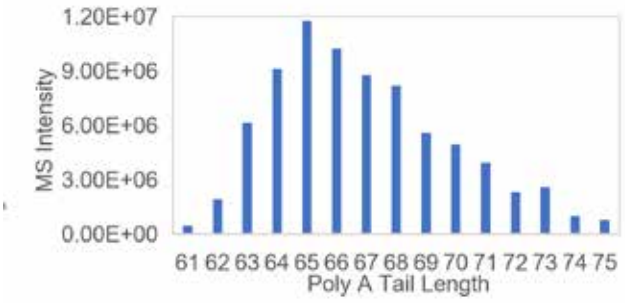




We have developed optimal conditions for 5' end cleavage and separation of 5' end oligonucleotides, allowing for accurate separation of capped and uncapped fragments.

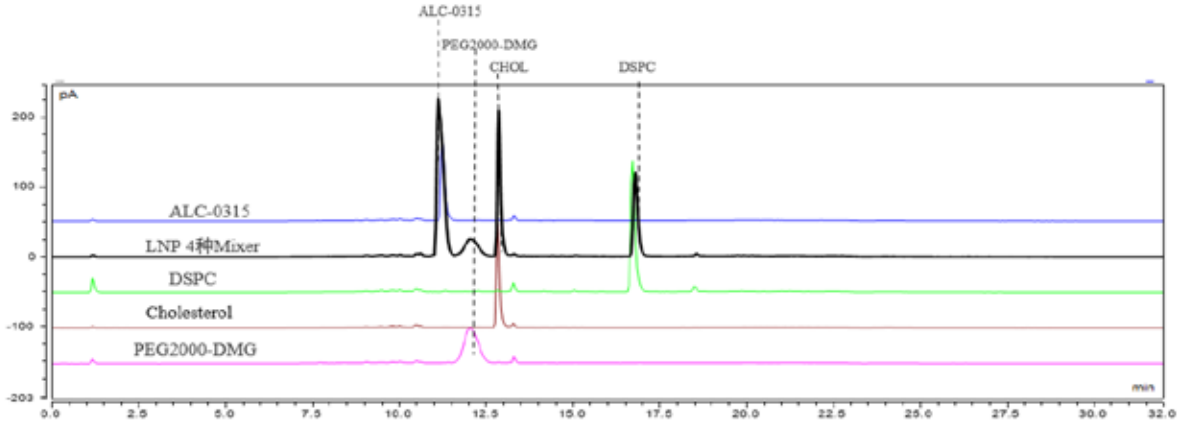
Detection of mRNA PolyA Tail Distribution (LC-MS)





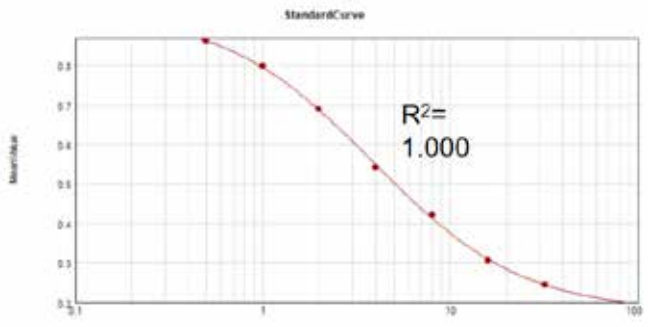
We have developed optimal conditions for the cleavage of 3' ends and separation of 3' end oligonucleotides, which enable precise detection of the distribution of polyA tails.

LNP Component and Content Detection (HPLC)



We have established a suitable chromatographic method that achieves baseline separation of four LNP components. This method demonstrates excellent reproducibility.

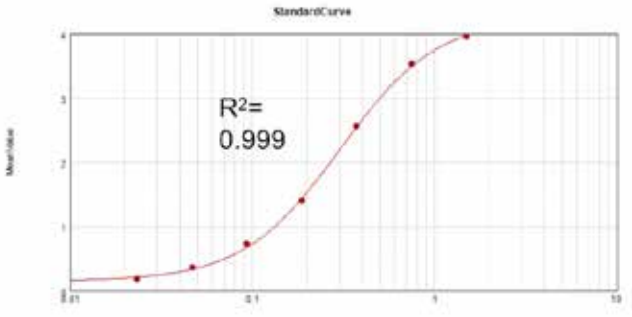
Residual Kanamycin Concentration (ELISA)



ConC (ng/ml)

Based on a commercial assay kit, we obtained a suitable calibration curve (R2 = 1.000) and achieved a recovery rate of 104.8%.

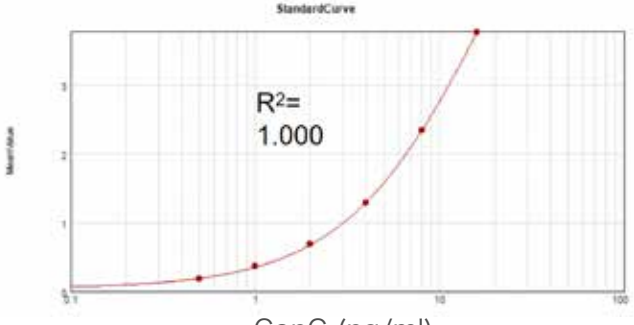
Residual dsRNA Concentration



ConC (ng/ml)

Based on a commercial assay kit, we obtained a suitable fitting calibration curve (R2 = 0.999) and achieved a recovery rate of 105.5%.

Residual T7 RNA Polymerase (ELISA)



ConC (ng/ml)

Based on a commercial assay kit, we obtained a suitable fitting calibration curve (R2 = 1.000) and achieved a recovery rate of 107.9%.

Residual Vaccinia Virus Capping Enzyme (ELISA)

ConC (ng/ml)

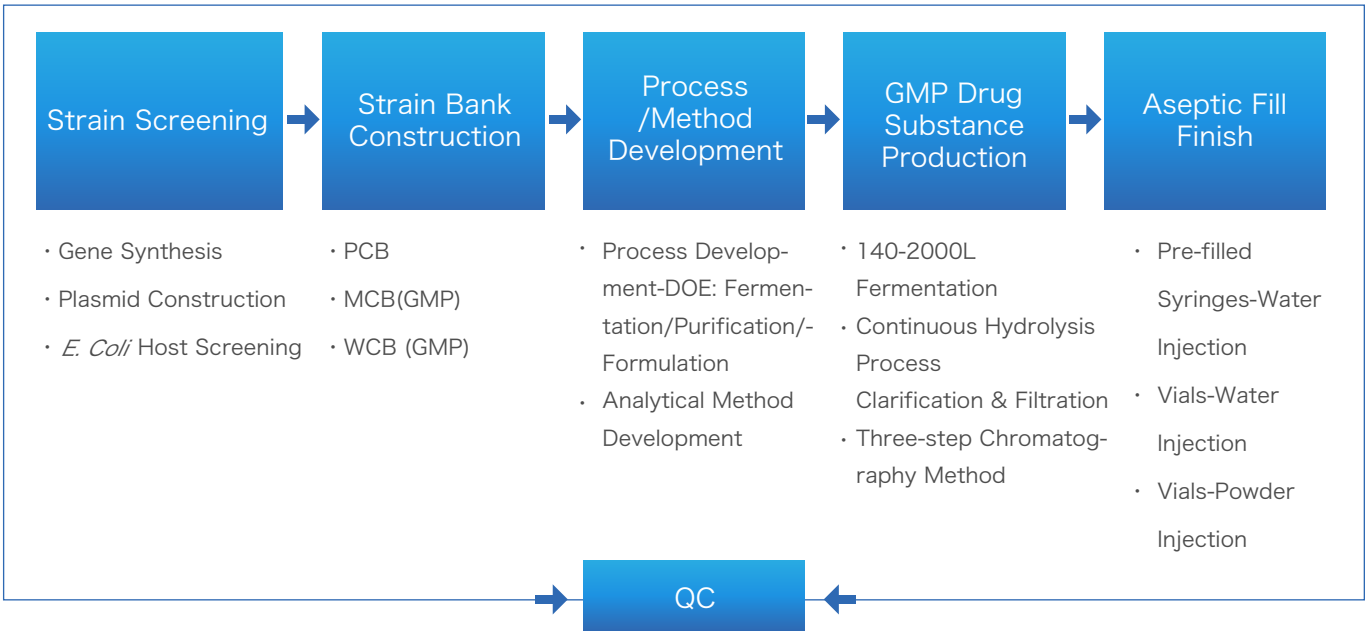
Based on a commercial assay kit, we obtained a suitable fitting calibration curve (R2 = 1.000) and achieved a recovery rate of 92%.

DNA Vaccine CDMO Services

DNA vaccines and mRNA vaccines share similarities in that both can encode any antigen related to pathogenic microorganisms or tumors, and can stimulate the immune response without the need for viral vectors or adjuvants. However, in terms of structure, DNA vaccines are more stable than mRNA vaccines. In addition to their use in infectious disease prevention, DNA vaccines have also accumulated rich clinical experience in the field of tumor therapy. DNA vaccines have a significant market application in both human and veterinary vaccine fields.

Yaohai Bio-Pharma, with its powerful process development platform and extensive experience in plasmid DNA production, can provide customers with a one-stop solution from plasmid DNA strain development to GMP production. We flexibly adjust the service process according to the customized needs of customers and provide high-quality DNA drug substance (DS) or drug product (DP) in quantities ranging from ten grams to hundreds of grams, as well as complete development and GMP production records and testing reports.

One-stop Solution for Plasmid DNA



Product Grade	Deliverable Products	Deliverable Specifications	Sample Applications
non-GMP	Drug Substance of Plasmid DNA	0.2~10 g	Analytical method development Stability Pre-experiments Formulation Development
	Drug Product of Plasmid DNA		
GMP, Sterility	Drug Substance of Plasmid DNA	10~100 g	IND/CTA Clinical Trial Samples Commercialized Products
	Drug Product of Plasmid DNA	(Water Injection/ Powder Injection)	

Plasmid CDMO Services, Covering the Entire Lifecycle of mRNA

Discovery, Research → IND/CTA → Phase 1/2 → Phase 3 → BLA/MAA → Commercial

Early Research

Strain Development

- Gene synthesis
- Plasmid construction（Clear and Transparent Traceability）
- *E. coli* host strain/ cell screening
- Establishment and Validation of a Tier 3 Microbial Strain Bank

Process Development and Optimization

Process Development and Optimization

- Fermentation Process: Media Components, pH/Temperature, Feeding Strategy
- Purification Process: Alkaline Lysis, RNA Removal, Supercoiling Capture, Endotoxin Removal
- Drug Product Process: Formulation Development, Process Development

Process Characterization and Process Validation, Process Performance Qualification (PPQ)

Analytical Method Development and Validation

- Method Development and Validation (Intermediate, Drug Substance, Drug Product, Impurities)
- Control of Critical Quality Attributes: Identification, Integrity, Purity
- Pre-stability Studies, Drug Substance and Drug Product Stability Studies

Technology Transfer and GMP production

- Technology Transfer
- Raw Material Testing and Release
- GMP Drug Substance, Aseptic Formulation Registered Batch Production
- GMP Drug Substance, Aseptic Formulation Clinical Sample Production
- GMP Drug Substance, Aseptic Formulation Commercial Product Production
- QA and QC

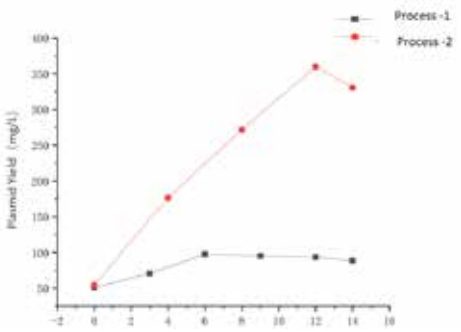
- Preparation of CTD Format Regulatory Submissions
- Global Registration Services

Platform Features

Plasmid DNA Fermentation Technology

- With high density fermentation, the plasmid DNA yield reached 350 mg/L under Yaohai platform process
- No animal sources, no antibiotics added or use of antibiotics that meet regulatory requirements Based on QbD and DoE concept, quickly identify the influencing factors to achieve process development goals
- After 2 to 3 batches of confirmation, the pilot process is amplified step by step to reduce the risk of process scale-up and process transfer.

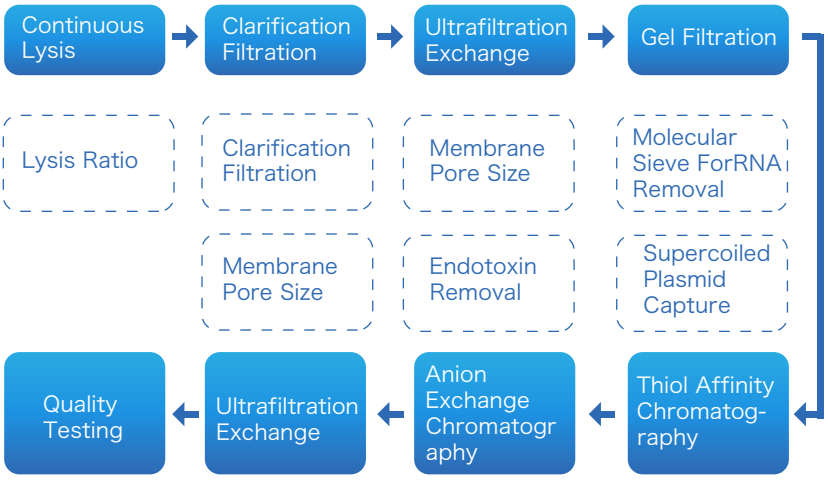
Vaccine Type	R&D Phase	Development Objectives	Deliverables
Therapeutic DNA Vaccine	pre IND	Fermentation process development: Focus on plasmid DNA yield	Using Yaohai platform'sbatch fermentation process, the yield offermentative liquid plasmid reached 350mg/L; further optimization can becarried out on this basis.



Plasmid DNA Purification Process

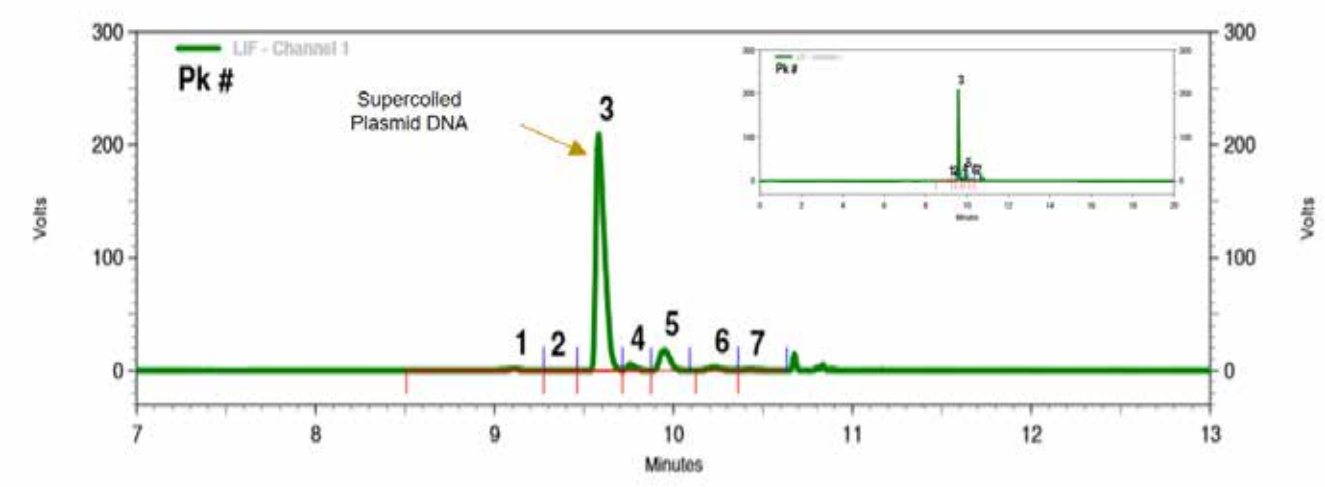
- We formulate development and production strategies based on the complexities of the project to meet the key quality attributes of the product while enhancing plasmid recovery.
- We have established a stable and scalable continuous cleavage process, as well as a three-step chromatography process that can efficiently capture supercoiled plasmids and effectively eliminate RNA, HCP, HCD, and endotoxins.

Vaccine Type	R&D Phase	Process Difficulty	Delivery
Prophylactic DNA Vaccine	pre IND	• Under the original process of thecustomer,HCD exceeded thestandard, and the proportion ofplasmid superhelix was about80%. • Development Objectives: Control HCD < 1% The proportion of superhelix plasmidwas > 90%	Yaohai team optimized the purification processaccording to the key indicators. Test results of a small batch of confirmed samples: • The proportion of superhelix was > 95% • The HCD residue was < 1% • HCP and endotoxin residues met the quality standards



Analytical Method Development

- We follow guidelines such as ICH, Chinese Pharmacopoeia (Chp), and United States Pharmacopeia (USP), and establish comprehensive method development, validation, and confirmation strategies based on product use and quality characteristics.
- Our development projects include ultra-supercoiled plasmid purity (HPLC/CE), HCD, HCP, residual RNA, residual antibiotics, etc., with considerations covering specificity, linearity/range, accuracy, precision, robustness, etc.



We have developed a plasmid DNA analysis protocol based on capillary gel electrophoresis with laser-induced fluorescence detection (CGE-LIF). This method effectively separates plasmid DNA of various conformations with high resolution and good reproducibility.

Platform Features

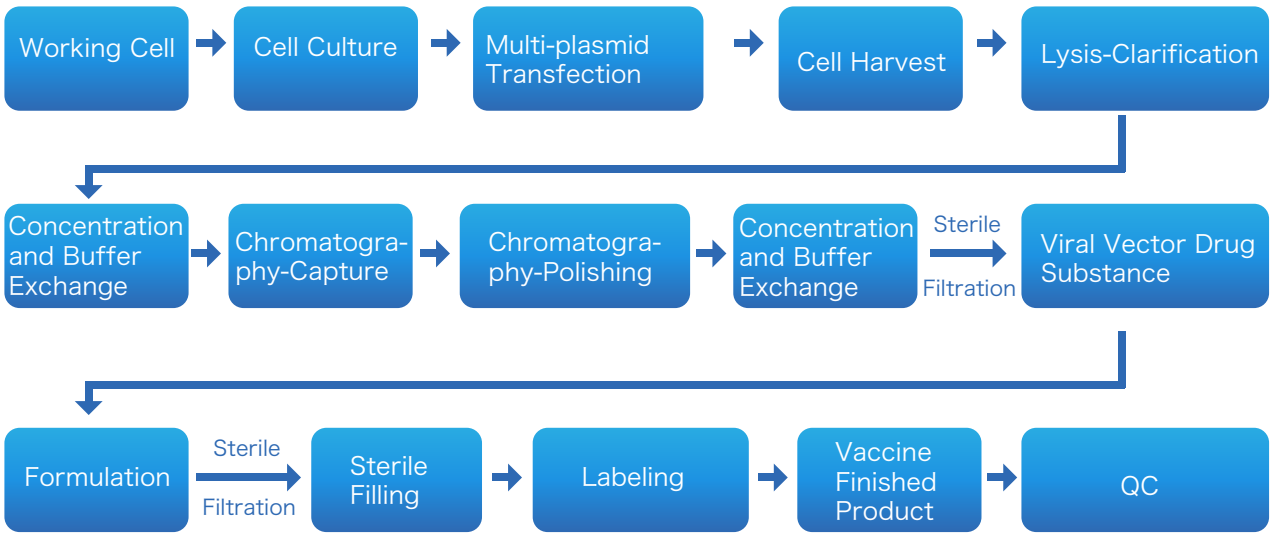
Viral Vector Vaccine CDMO Services

In addition to DNA vaccines that utilize naked plasmids as carriers, researchers have also developed viral vector vaccines. Viral vector vaccines typically employ a harmless virus, such as adenovirus, adeno-associated virus, lentivirus, or herpes simplex virus, as the carrier. The target DNA sequence is integrated into the viral vector, allowing for the expression of the target antigen within the body and subsequent activation of the immune response.

Recombinant viral vectors are created by using plasmid DNA encoding the target antigen gene as the raw material, which is then transfected into cells and packaged into viruses. Leveraging a robust process development platform and extensive experience in plasmid DNA production, Yaohai Bio-Pharma offers customers efficient solutions for GMP-grade plasmid DNA production, GMP-grade viral vector vaccine production, and sterile filling. We are dedicated to meeting the specific needs of our clients, and can adjust our service process accordingly. Our services include the provision of DNA raw materials and viral vector vaccine drug substances (DS) at a scale of tens of grams, as well as comprehensive development and production reports and testing reports.

One-stop Solution for Viral Vector Vaccines

Using plasmid DNA as the raw material, the details of the service can be found in the [DNA Vaccine CDMO Services] section.

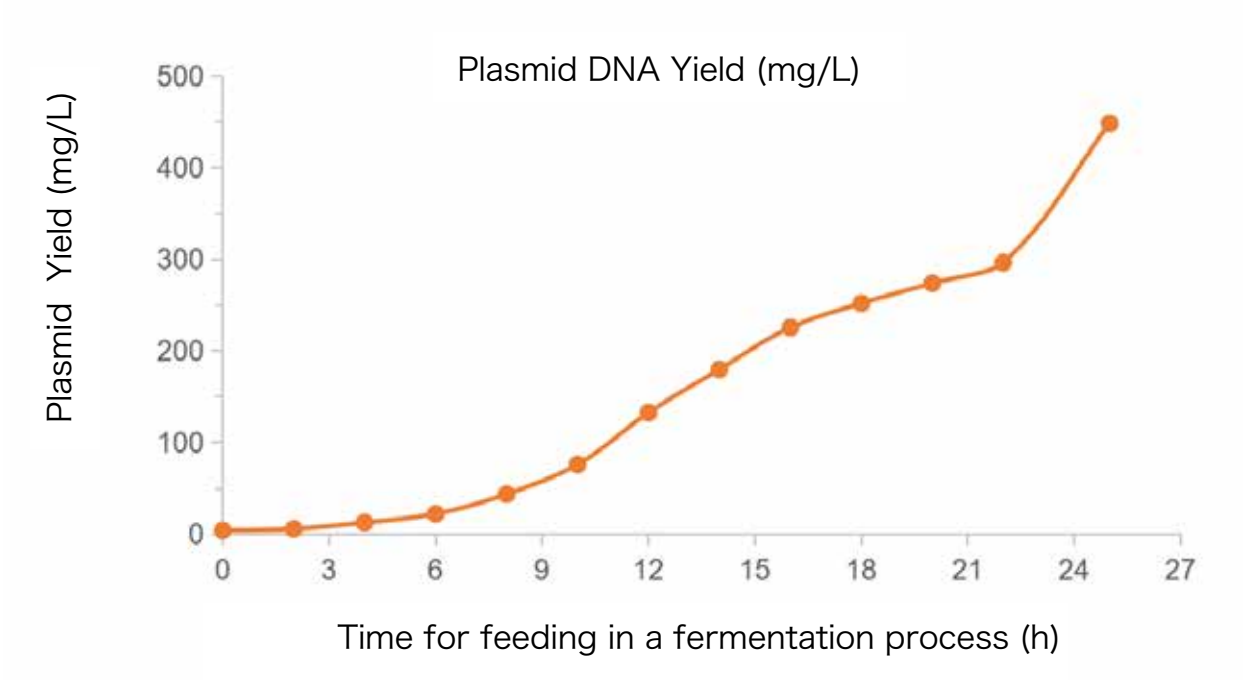


Product Grade	Deliverable Products	Deliverable Specifications	Sample Applications
non-GMP	Drug Substance of Viral Vector Vaccine	Customization	Method Development Stability Pre-experiment Formulation Development
	Drug Product of Viral Vector Vaccine		
GMP, Sterility	Drug Substance of Viral Vector Vaccine	Customization	IND/CTA Filing Clinical Samples Commercialized Products
	Drug Product of Viral Vector Vaccine	Water Injection Powder Injection	

Case Study

- High-density fermentation, plasmid yield reaches 450 mg/L
- Fermentation process is animal-free, complying with regulatory requirements for the use of antibiotics (preferably kanamycin)

Vaccine Type	R&D Phase	Development Objectives	Deliverables
Viral Vector Vaccine	pre IND	Fermentation process development: Enhancing plasmid supercoiling ratio to maintain high plasmid DNA yield.	Plasmid DNA yield in the fermentation broth is approximately 450 mg/L; The plasmid supercoiling ratio exceeds the client's expectations.



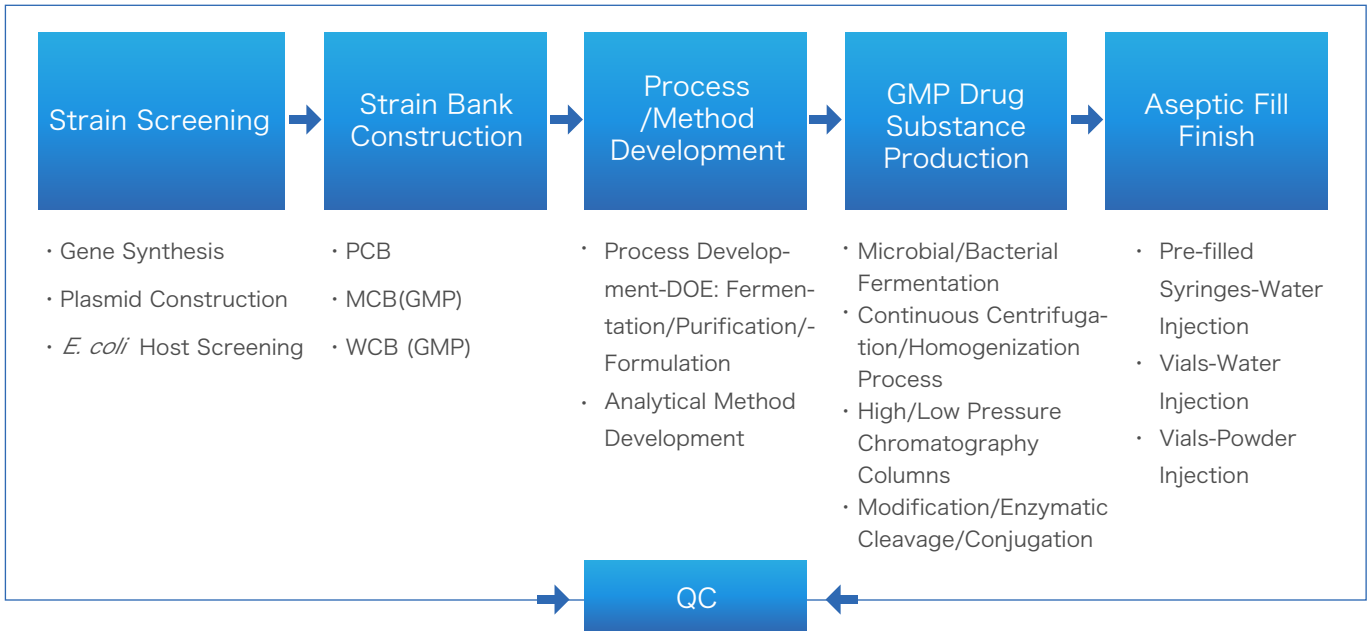
Recombinant Subunit Vaccine CDMO Services

Recombinant subunit vaccines are produced using DNA recombination technology to express target antigens in hosts such as *E. coli* yeast, and animal cells. Recombinant subunit vaccines have been used to prevent various diseases, including SARS-CoV-2, HBV, HPV, RSV, VZV, and Neisseria meningitidis.

Virus-like particles (VLPs) are a type of recombinant subunit vaccine in which single or multiple antigens self-assemble to form VLPs that stimulate the body's immune response.

Based on the "recombinant protein service platform," Yaohai BioPharma provides a one-stop solution for customers from strain development to GMP production of recombinant subunit vaccines. We can flexibly adjust the service process according to customers' customized needs, providing customers with kilogram or ten-gram level recombinant subunit vaccine drug substance (DS) or drug products (DP), as well as process development and GMP production records and testing reports.

One-stop Solution for Recombinant Subunit Vaccines



Product Grade	Deliverable Products	Deliverable Specifications	Sample Applications
non-GMP	Drug Substance of Recombinant protein	0.2~10 g	Preclinical studies, Analytical method development, stability pre-experiments, formulation development
	Drug Product of Recombinant protein		
GMP, Sterility	Drug Substance of Recombinant protein	2~100 g	IND/CTA, clinical samples, commercialized products
	Drug Product of Recombinant protein	(Water Injection/ Powder Injection)	

Recombinant Subunit Vaccine CRDMO Service, Covering the Entire mRNA Lifecycle

Discovery, Research → IND/CTA → Phase 1/2 → Phase 3 → BLA/MAA → Commercial

Early Research

Strain Development

- Gene Synthesis
- Plasmid Construction（Clear and Transparent Traceability)
- *E. coli* and Yeast Host Strain/ Cell Screening
- Establishment and Validation of a Tier 3 Microbial Strain Bank
  - PCB
  - MCB (GMP)
  - WCB (GMP)

Process Development and Optimization

Process Development and Optimization

- Fermentation Process： Media Components, pH/Temperature, Feeding Strategy
- Purification Process: Target protein capture, HCP/HCD/Endotoxin Removal, VLP disassembly and reassembly
- Drug Product Process: Formulation Development, Process Development

Process Characterization and Process Validation, Process Performance Qualification (PPQ)

Analytical Method Development and Validation

- Method Development and Validation (Intermediate, Drug Substance, Drug Product, Impurities)
- Control of Critical Quality Attributes: Identification, Integrity, Purity
- Pre-stability Studies, Drug Substance and Drug Product Stability Studies

Technology Transfer and GMP production

- Technology Transfer
- Raw Material Testing and Release
- GMP Drug Substance, Aseptic Formulation Registered Batch Production
- GMP Drug Substance, Aseptic Formulation Clinical Sample Production
- GMP Drug Substance, Aseptic Formulation Commercial Product Production
- QA and QC

- Preparation of CTD Format Regulatory Submissions
- Global Registration Services

Case Study

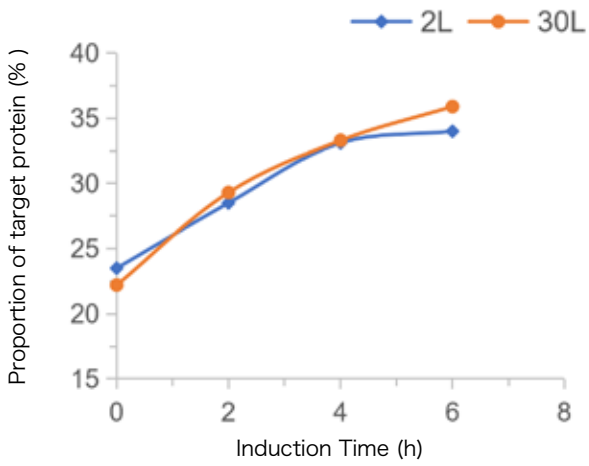
Case 1: Recombinant Subunit Vaccine

- Our professional project management expertise in fermentation, purification, formulation, and analytical methods transfer enables team members to identify and control project risks, and promote project operation throughout the entire cycle.
- The product's analytical methods and quality standards comply with ICH, Chinese, and Australian regulatory requirements.
- We guarantee a single project operation system in the GMP workshop, which effectively prevents pollution and confusion. After passing the cleaning validation, we proceed to the next project.
- We follow a compliant GMP quality management system, where people, machines, materials, methods, and the environment are under control during production activities.

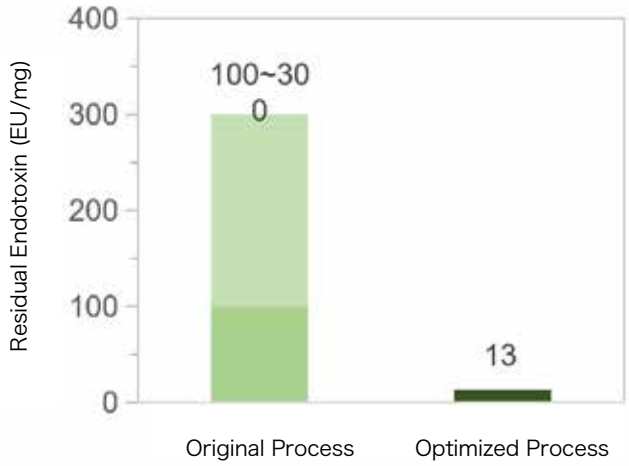
Vaccine Type	R&D Phase	Customer's Need	Deliverables
RSV Vaccine *(Escherichia coli)*	pre IND	Identify and control technology transfer risks to achieve a stable production process. Conduct raw material and formulation production activities in the GMP workshop, delivering 3000 units of sterile penicillin vial formulations.	· 3000 units of sterile penicillin vial formulations (containing grade-g recombinant protein). · The product's Certificate of Analysis (COA), process specifications, quality standards, and production records comply with the GMP system.

Case 2: Recombinant VLP Vaccine

Vaccine Type	R&D Phase	Customer's Need	Deliverables
VLP Vaccines (Encapsulating nucleic acid)	pre IND	· Optimize the fermentation process to increase the proportion of the target protein. · In the original purification process, the endotoxin level was between 150-300 EU/mg. The goal is to reduce the endotoxin level to below 150 EU/mg.	Yaohai has optimized the process for key indicators and successfully scaled it up to 30L and 200L. The intracellular proportion of the target protein is greater than 35%. The protein purity is greater than 98% (HPLC), while preserving intact nucleic acids. The residual endotoxin level is less than 13 EU/mg.



The 2L small-scale process was successfully scaled up to a 30L pilot scale, and the expression ratio of the target protein remained relatively unchanged after scaling up.



After purification process optimization, the endotoxin residue is less than 13 EU/mg.

Protein/Peptide Therapeutic Vaccine CDMO Services

In addition to recombinant subunit vaccines targeting pathogen antigens, researchers have focused on targeting proteins in tumor cells or other metabolic pathway-related antigens. These antigens can stimulate the body to produce specific antibodies that kill tumor cells or block target metabolic pathways, achieving the goal of treating diseases. Based on a comprehensive "recombinant protein service platform," Yaohai Bio-Pharma can provide customers with a one-stop solution from strain development and protein sample preparation to GMP production of recombinant protein vaccines. We can flexibly adjust the service process according to the customer's customized needs, providing customers with high-quality recombinant protein Drug Substance (DS) or Drug Product (DP) in grams or tens of grams, as well as process development and GMP production records, and testing reports.

One-stop Solution for Protein/Peptide Therapeutic Vaccines

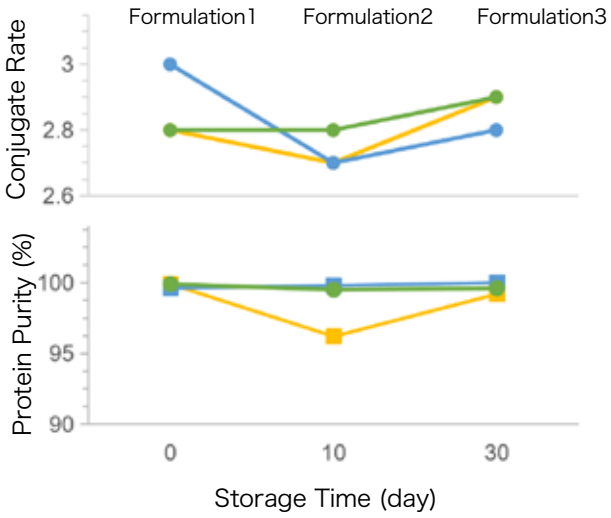
The recombinant protein/peptide therapeutic vaccine services offered by Yaohai Bio-Pharma are also based on the [recombinant protein service platform]. For more details about the service, please refer to the "Recombinant Subunit Vaccine CDMO Services".

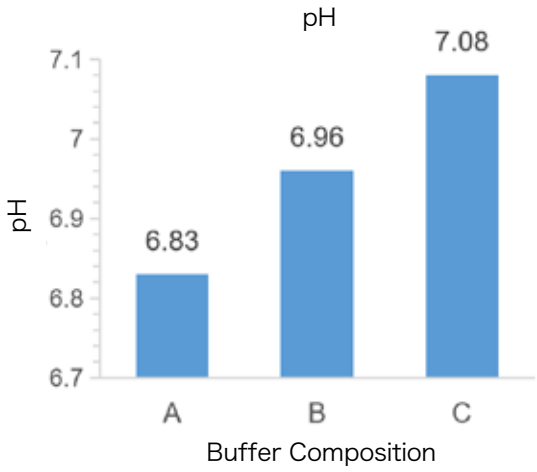
<table>
<tr><th>Product Grade</th><th>Deliverable Products</th><th>Deliverable Specifications</th><th>Sample Applications</th></tr>
<tr><td rowspan="2">non-GMP</td><td>Drug Substance of Recombinant protein</td><td rowspan="2">0.2~10 g</td><td rowspan="2">Preclinical studies, Analytical method development, stability pre-experiments, formulation development</td></tr>
<tr><td>Drug Product of Recombinant protein</td></tr>
<tr><td rowspan="2">GMP, Sterility</td><td>Drug Substance of Recombinant protein</td><td>2~100 g</td><td rowspan="2">IND/CTA, clinical samples, commercialized products</td></tr>
<tr><td>Drug Product of Recombinant protein</td><td>(Water Injection/ Powder Injection)</td></tr>
</table>

Case Study

Vaccine Type	R&D Phase	Customer's Need	Deliverables
Recombinant protein therapeutic vaccine	pre IND	· Control technology transfer risk and obtain a stable raw material production process. · Deliver G-grade recombinant protein raw materials. · Ensure production activities comply with all GMP specifications.	· Delivery of recombinant protein raw material that meets quality standards. · Delivery of raw material COA, process specifications, quality standards, production records, and other documents that fully comply with the GMP system
Therapeutic vaccine with VLP as carrier	pre IND	· Drug substance: Coupling of antigen-VLP carrier protein is performed in GMP workshop. · Drug product: Prescription development and sterile filling.	· Delivery of stable raw material formulation and formulation recipe (including adjuvants) and scalable formulation process. · Coupling production is in progress

Note: Yaohai also provides one-stop solutions for VLP carriers, the details of the service can be found in the [Carrier Protein CDMO Services]





Recombinant Carrier Protein CDMO Services

Conjugating the target antigen with a carrier protein is a strategy used in vaccine development. There are currently marketed products known as conjugate vaccines and polysaccharide conjugate vaccines. The carrier proteins approved for use are primarily derived from pathogenic microorganisms, considering production yield and safety. Scientists are investigating possibly utilizing DNA recombinant technology to create carrier proteins. This includes non-toxic mutant CRM197 of diphtheria toxin, tetanus toxin (TT), and Neisseria meningitidis P64k protein. Additionally, novel VLP carrier vaccines are also being developed.

Yaohai Bio-Pharma offers a comprehensive recombinant protein service platform that provides customers with a complete solution. This includes strain development and GMP production of recombinant carrier proteins. We can deliver carrier proteins ranging from gram to ten-gram scale, meeting quality specifications. We also provide relevant records and reports tailored to the specific needs of our customers.

Types	Name of Carrier Proteins	Types of Strain	Production Platform
Recombinant Protein	carrier VLP	・*Escherichia coli* ・Yeast ・Other prokaryotic/eukaryotic microorganisms, suspension cells, adherent cells	・Microbial/suspension cell/adherent cell fermentation system ・Centrifugation and homogenization equipment High/low-pressure chromatography system ・Conjugation reaction vessel GMP quality system
	Diphtheria toxin non-toxic mutant		
	Tetanus toxin (TT)		
	Neisseria meningitidis P64k protein		
	Pseudomonas aeruginosa exotoxin A (EPA)		
	Other recombinant carrier proteins		

Other related services: Yaohai Pharma is equipped with BSL-2 bio-safety level workshops and provides carrier protein solutions based on pathogenic bacteria. For more information, please refer to the [Non-Recombinant Carrier Protein CDMO Services].

One-stop Solution for Recombinant Carrier Proteins

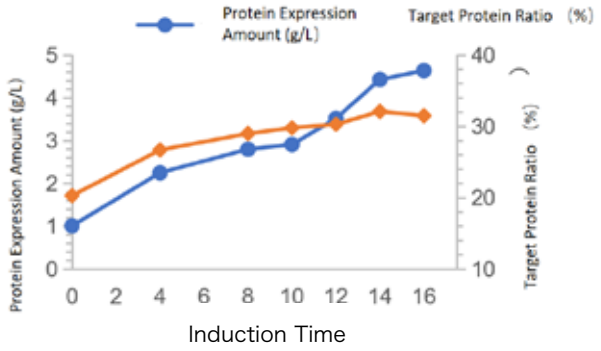
Yaohai Bio-Pharma recombinant carrier protein service is based on the [Recombinant Protein Service Platform]. For more information, please refer to the [Recombinant Subunit Vaccine CDMO Services].

Product Grade	Deliverable Products	Deliverable Specifications	Sample Applications
non-GMP	Drug Substance of Recombinant protein	0.2~10G	Preclinical studies, Analytical method development, stability pre-experiments, formulation development
	Drug Product of Recombinant protein		
GMP, Sterility	Drug Substance of Recombinant protein	2~100G	IND/CTA, clinical samples, commercialized products
	Drug Product of Recombinant protein	20,000~60,000 (Pre-fill and Finish/ Vials)	

Case Study

Case 1: Recombinant VLP Carrier Vaccine

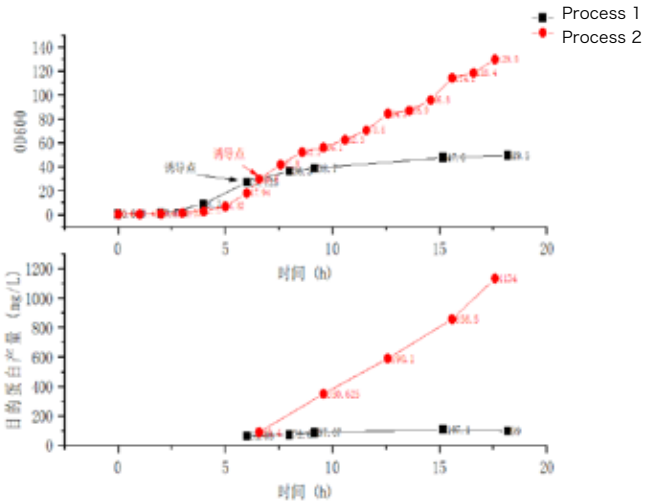
Vaccine Type	R&D Phase	Customer's Need	Deliverables
VLP Carrier Vaccine *(Escherichia coli)*	pre IND	· Process Development: Fermentation, Purification · Process Scale-up and Technology Transfer · GMP Production: G-grade Carrier Protein that meets quality	· Stable small-scale process, successfully scaled up to GMP production · VLP carrier expression level reaches 4 g/L · Protein purity, endotoxin, and other impurities meet quality standards · Delivery of G-grade recombinant protein and COA documentation



Fermentation process development of VLP carrier protein:
Protein expression amount exceeds 4 g/L, with a target protein ratio of over 30%.
The process has been successfully scaled up to GMP production scale with stability.

Case 2: Recombinant CRM197 Carrier Protein Vaccine

Vaccine Type	R&D Phase	Customer's Need	Deliverables
Recombinant CRM197 Protein Vaccine *(Escherichia coli)*	Preclinical	non GMP Research sample preparation · Total amount: 40 mg · Purity: > 90%	· Based on the characteristics of the strain and protein physicochemical properties, Yaohai BioPharma designed the process route. · We have developed a fermentation process, where the target protein is expressed in soluble form, with a yield of 1134 mg/L. · Purification process development in progress.



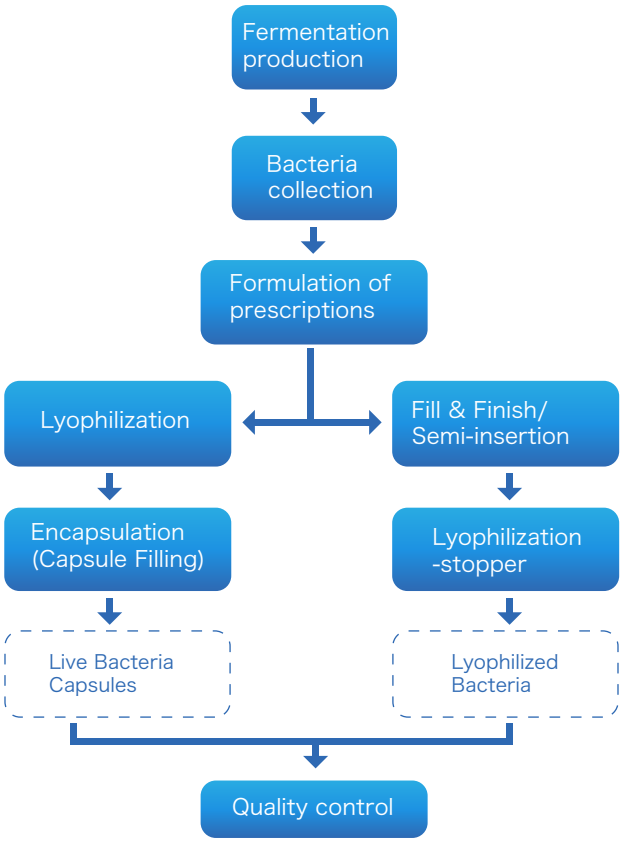
Fermentation process development for the expression of recombinant CRM197 in *E. coli*. Initially, we designed two fermentation processes: Process 1 and Process 2, which showed significant differences in bacterial growth and protein expression. By using Process 2, the recombinant protein expression level reached 1134 mg/L.

Live-attenuated Vaccines (Bacteria) CDMO Services

Live-attenuated vaccine is a mature vaccine development strategy, generally involving natural weak strains, artificially passaged selected strains, or genetically modified attenuated strains. These vaccines maintain their immunogenicity and stimulate immune responses in the body, effectively preventing diseases. Live attenuated vaccines have been successfully utilized for the prevention of viral or bacterial infections, including human typhoid Salmonella live vaccine, attenuated cholera vaccine, as well as veterinary vaccines like Bordetella bovis vaccine, Bartonella multocida porcine pleuropneumonia live vaccine, and piglet paratyphoid live vaccine.

Yaohai Bio-Pharma has over ten years of experience as a microbial CDMO, providing Contract Development and Manufacturing services for live attenuated bacterial vaccines. Our Biosafety Level 2 (BSL-2) operational area ensures the highest level of safety during microbial strain development, GMP drug production, and aseptic Fill & Finish. We offer customized solutions tailored to the unique requirements of our clients, delivering bacterial body (DS, API) or live bacterial drug product that meet the highest quality standards. Our GMP production records and testing reports provide our clients with complete transparency and confidence in our services.

Production Process



Fermentation Parameter: Temperature, pH, Dissolved Oxygen,Filler, Fermentation Time

e.g.: Continuous-flow Centrifugation, Feeding Speed, Rotation Speed, Discharge Time

Prescription Formulation, Components of Buffer Solution,
Components of Lyoprotectant

Types of Raw Materials and Packaging Materials
Freeze-drying Process, Can sealing or Filling Process

Samples: Raw Materials, Packaging Materials, Bacterial Liquid,
Lyophilized Bacterial Liquid, Finished Products

Test: Appearance, pH, Microscopic Examination, Viable Bacteria Count,
Potency, Impurities, etc.

Process for the preparation of live attenuated human vaccines (capsule vs. lyophilized bacteria)

Delivery

Product Grade	Deliverable Products	Form of Delivery	Sample Applications
GMP	Bacterial Body Drug Substance	Bacterial Fluid	· IND/CTA · Clinical Samples · BLA/MAA · Commercialized Products
	Live Bacterial Drug Product	Capsules Bacteria Suspension Lyophilized Bacteria Other Dosage Forms	

Live Bacterial Vector Vaccine CDMO Services

The design concept of microbial vector (live bacterial vector) vaccines is to modify attenuated pathogens or symbiotic bacteria based on genetic engineering technology to deliver target antigens and activate the body's immune response. The greatest advantage of live bacterial vectors is that they can stimulate a wide range of humoral immunity and cellular immunity. The development direction of microbial vector vaccines includes the prevention of infectious diseases and the treatment of tumors.

Yaohai Bio-Pharma has more than ten years of microbial CDMO experience. We have established a GMP workshop with biosafety levels BSL-1 and BSL-2, and launched a one-stop solution for microbial vector vaccine CDMO, covering from microbial strain development to GMP production. Based on the customized needs of customers, we provide customers with bacterial bodies drug substance (DS, API) or live bacterial drug product (DP) that meet quality standards, as well as GMP production records and test reports.

Microbial Vector Vaccine Preparation Process

Microbial vector vaccines are also live bacterial preparations, and their preparation process is the same as the [attenuated live vaccine preparation process].

Delivery

<table>
<tr><th>Product Grade</th><th>Deliverable Products</th><th>Form of Delivery</th><th>Sample Applications</th></tr>
<tr><td rowspan="2">GMP</td><td>Bacterial Body</td><td>Bacterial Fluid</td><td rowspan="2">IND/CTA
Clinical Samples
BLA/ MAA
Commercialized Products</td></tr>
<tr><td>Live Bacterial Drug Product</td><td>Freeze-dried Bacterial Body</td></tr>
</table>

Inactivated Vaccine (Bacteria) CDMO Services

Pathogenic microorganisms are inactivated by physical or chemical methods to make inactivated vaccines. Inactivated vaccines lose their pathogenicity but retain good immunogenicity. Inactivated vaccines have been used to prevent viral or bacterial infections, such as the human typhoid Salmonella inactivated vaccine, as well as animal vaccines such as the swine erysipelas inactivated vaccine, piglet *Escherichia coli* inactivated vaccine, Haemophilus parasuis inactivated vaccine, multi-kill Bacillus inactivated vaccine, and swine bronchial septicemia Bordetella inactivated vaccine.

Yaohai Bio-Pharma has more than ten years of microbial CDMO experience. Based on the BSL-2 operation area, we provide a one-stop solution from microbial strain development to GMP production of inactivated vaccines.

Based on the customized needs of customers, we provide customers with inactivated vaccine drug substance (DS, API) or drug product (DP) that meet quality standards, as well as GMP production records and test reports.

Inactivated Vaccine (Bacteria) CDMO Services

Inactivated Vaccine Drug Product Process

Fermentation Production	Fermentation Parameters: Temperature, pH, Dissolved Oxygen, Feed, Fermentation Time
↓	
Inactivation	Example: Formaldehyde Inactivation: Formaldehyde Concentration, Treatment Time
↓	
Formulation Preparation	Formulation Preparation: Buffer Solution, Stabilizer, Adjuvant, etc.
↓	
Filling - Stoppering	Filling Method: Ordinary, Nitrogen Filling, Vacuum
↓	
Vaccine Finished Product	Samples: Raw Materials, Packaging Materials, Intermediate Products, Drug Substance, Finished Product
↓	
Quality Control	Test Items: Appearance, pH, Sterility, Formaldehyde, Safety, Potency

Drug Product Process of Bacterial Inactivated Vaccine (Water Injection)

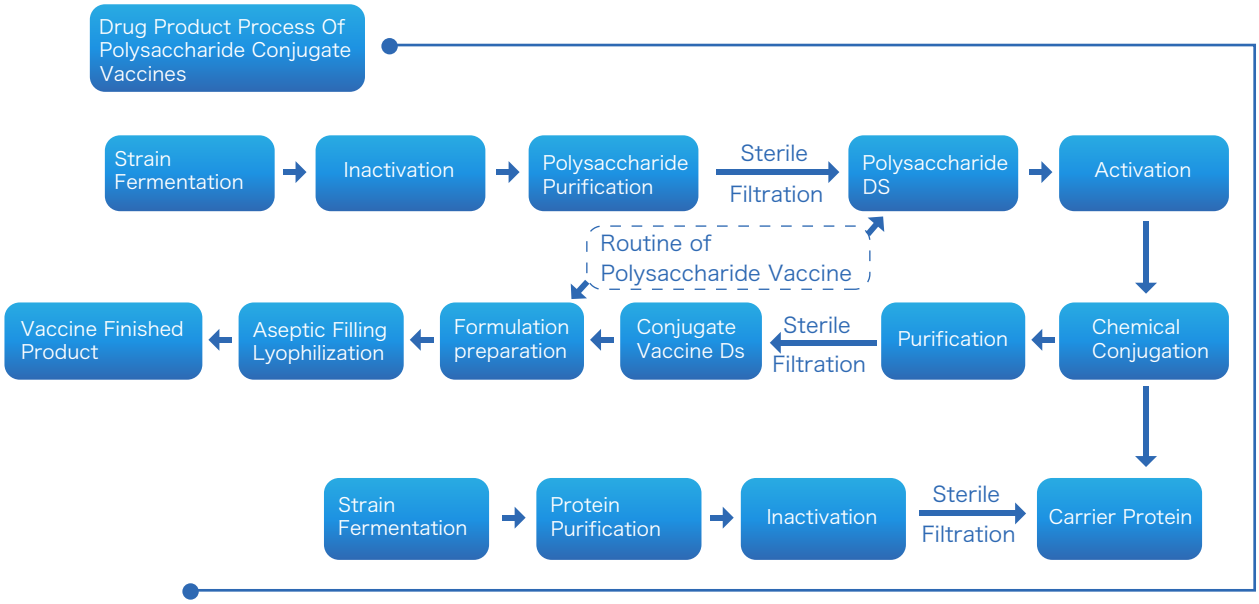
Delivery

Product Grade	Deliverable Products	Form of Delivery	Sample Applications
GMP	Vaccine DS	DS	· IND/CTA · Clinical Samples · BLA/ MAA · Commercialized Products
	Vaccine Finished Product	Water Injection Powder Injection Other Dosage Forms	

CDMO Services for Polysaccharide Vaccines or Conjugate Vaccines

Pathogenic bacteria such as Haemophilus influenzae type B, meningococcal, pneumococcal, and typhoid Salmonella have a capsular structure, which can cause invasive infections in children. Capsular polysaccharides are important factors causing these bacterial infections and are the target antigens for vaccine development. Vaccines based on bacterial polysaccharides include polysaccharide vaccines and conjugate vaccines. Polysaccharide vaccines use polysaccharide antigens as active ingredients. Conjugate vaccines are formed by coupling polysaccharides with carriers such as toxoids, which can enhance the protective effect of the vaccine. Yaohai Bio-Pharma has more than a decade of microbial CDMO experience. Based on the GMP workshop with a biosafety level of BSL-2, we provide a one-stop solution for microbial strain development, fermentation, extraction and purification of polysaccharides and carrier proteins, conjugation, and aseptic filling. According to the customized needs of customers, we provide customers with intermediates, vaccine drug substance (DS, API) or drug produce (DP) that meet quality standards, as well as GMP production records and test reports.

Drug Product Process of Polysaccharide Conjugate Vaccines



Delivery

Product Grade	Deliverable Products	Form of Delivery	Sample Applications
GMP	Raw Materials	Polysaccharide Antigen DS	· IND/CTA · Clinical Samples · BLA/MAA · Commercialized Products
		Carrier Protein DS	
	Vaccine DS	Conjugate Vaccine DS	
	Vaccine Finished Product	Water Injection Powder Injection Other Dosage Forms	

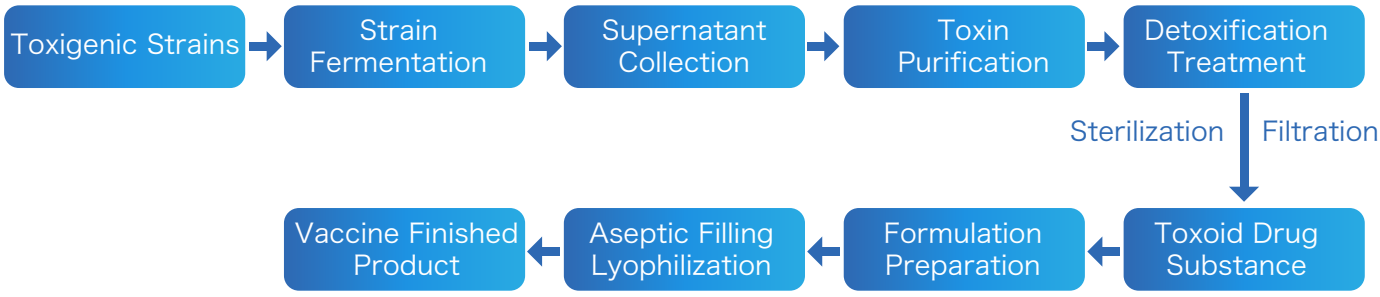
Toxoid Vaccine CDMO Services

Certain bacteria induce disease by secreting toxins, such as tetanus and diphtheria. Inactivated toxins prepared by physical and chemical methods are the main active ingredients of toxoid vaccines. Toxoid vaccines retain the immunogenicity that activates the production of specific antibodies but do not cause disease symptoms. Currently approved human toxoid vaccines include diphtheria and tetanus vaccines, and veterinary vaccines include multivalent Pasteurella toxoid and porcine pleuropneumonia Actinobacillus toxoid. Yaohai Bio-Pharma has over a decade of microbial CDMO experience. Based on the BSL-2 GMP workshop, we provide a one-stop solution for microbial strain development, fermentation, toxoid extraction and purification, and aseptic filling. According to the customized needs of customers, we provide clients with toxoid drug substance (DS, API) or vaccine drug product (DP) that meet quality standards, as well as GMP production records and test reports.

Some Approved Toxoid Vaccines Include:

Application	Toxoid name	Strain Type	Platform
Human	Diphtheria Toxoid (DT)	Corynebacterium Diphtheriae	· Microbial Fermentation Systems · Centrifugation, Homogenization Equipment · High/Low Pressure Chromatography Systems · Biosafety Level: BSL-2 · GMP Quality System
	Tetanus Toxoid (TT)	Clostridium Tetani	
Veterinary	Multivalent Pasteurella Toxoid	Multivalent Pasteurella	
	Hemolysin Toxins ApxI, ApxII, ApxIII	Actinobacillus Pleuropneumoniae	
Human and Veterinary	Other Toxin Proteins of Microbial Origin (BSL-1, BSL-2)		

Toxoid Vaccine Drug Product Process



Note: The order of operation for toxin purification and detoxification may vary depending on the type of toxin.

Delivery

Product Grade	Deliverable Products	Form of Delivery	Sample Applications
Gmp	Vaccine DS	DS	· IND/CTA · Clinical Samples · BLA/ MAA · Commercialized Products
	Vaccine Finished Product	Water Injection Powder Injection Other Dosage Forms	

Carrier Protein CDMO Services

Conjugation of the target antigen with carrier proteins is also a development strategy for vaccines, such as conjugate vaccines. Binding with carrier proteins can enhance the immunogenicity of vaccines. Currently, approved carrier proteins on the market include diphtheria toxoid (DT), non-toxic mutant of diphtheria toxin CRM197, tetanus toxoid (TT), etc.

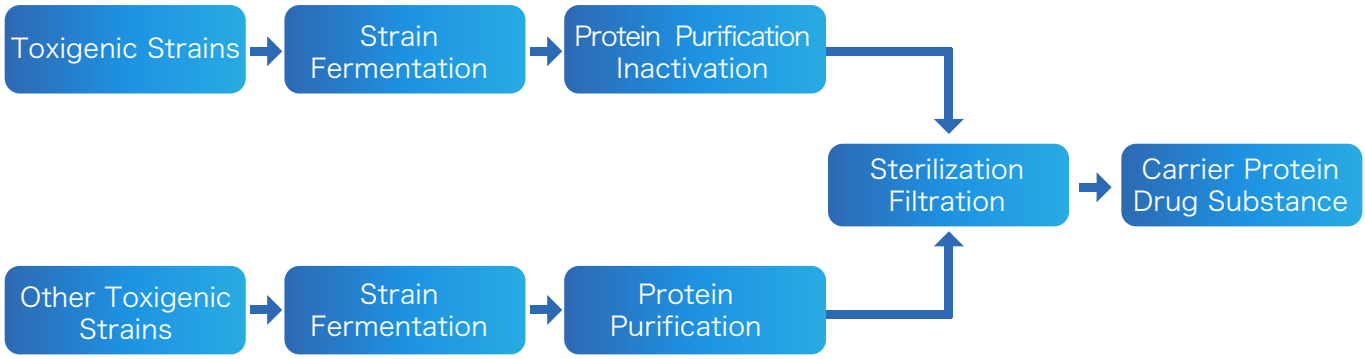
With a powerful process development platform, biosafety level BSL-1 and BSL-2 workshops, and a GMP quality system, Yaohai Bio-Pharma can provide customers with a one-stop solution from microbial strain development to GMP production of carrier proteins. We deliver gram to kilogram-scale carrier proteins that meet quality standards, as well as GMP production records and testing reports to our customers.

Partially Approved Carrier Proteins (Non-recombinant Proteins)

Protein Type	Carrier Protein Name	Strain Type	Platform
GMP	Diphtheria Toxin Non-toxic Mutants CRM197	Corynebacterium Diphtheriae	· Microbial Fermentation Systems · Centrifugation and Homogenization Equipments · High/Low Pressure Chromatography Systems · Conjugate Reaction Tank · Biosafety Level: BSL-2 · GMP Quality System
	Diphtheria Toxoid	Corynebacterium Diphtheriae	
	Tetanus Toxoid	Clostridium Tetani	
	Meningococcal Outer Membrane Protein Complex (OMPC)	Neisseria Meningitidis	
	other carrier proteins from microbial sources (BSL-1, BSL-2)		

Other services: Yaohai Bio-Pharma also provides recombinant carrier protein solutions based on microbial expression systems, for more details, please check it from [Recombinant Carrier Protein CDMO Services].

Carrier Protein Drug Product Process



Toxigenic strains: toxin-producing strains, such as diphtheria toxin, tetanus toxin;

Toxigenic strains: toxin-producing strains, such as diphtheria toxin, tetanus toxin;

Delivery

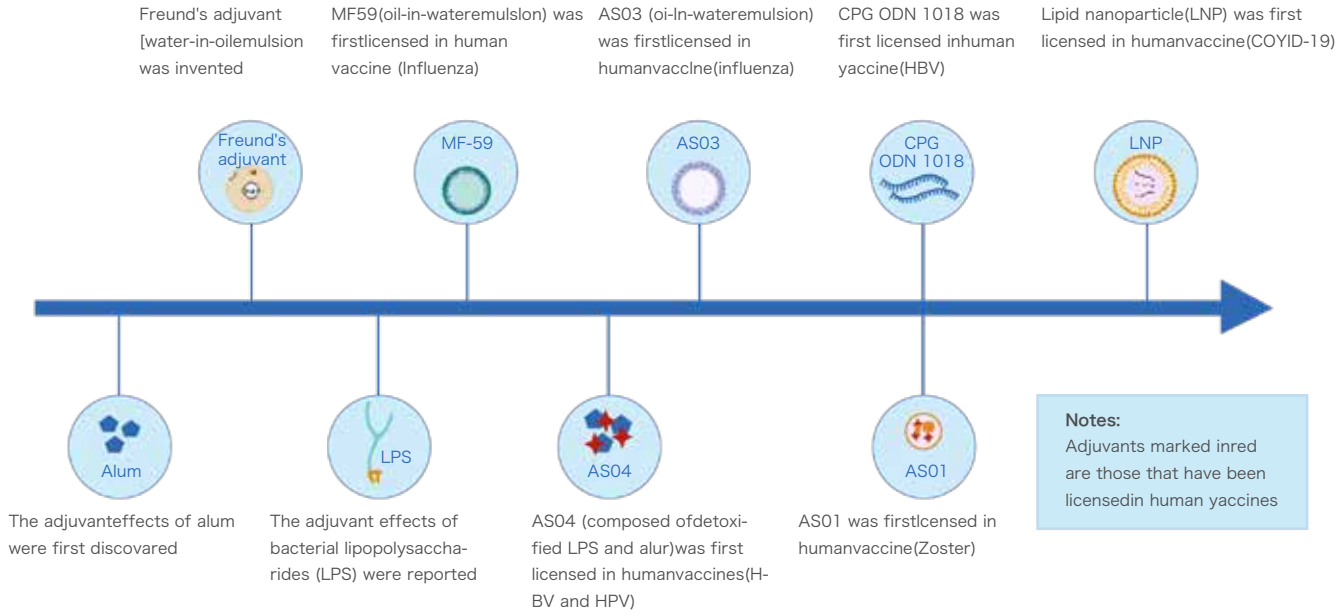
Product Grade	Deliverable Products	Form of Delivery	Sample Applications
GMP	Vaccine DS	DS	· Vaccine Production Raw Materials Combination
	Vaccine Finished Product	Lyophilized Powder	

Vaccine Adjuvants

Vaccinations are one of the most significant methods for preventing infectious diseases. The efficiency of a vaccination is determined not only by the antigen components, but also by adjuvants, which are frequently employed to boost the immune system more effectively. Adjuvants have various advantages, including reducing the amount of antigen per vaccine dose and the number of vaccination sessions, and in some situations, increasing the stability of the antigen component, extending its half-life and indirectly improving its immunogenicity.

Many other forms of adjuvants, such as mineral salts (Aluminum), emulsions (MF59, AS03), natural products (MPL, QS-21, Squalene), combined adjuvants (AS01, AS02), and cytokines (Interleukin, Interferons, GM-CSF), are now allowed for use in vaccine manufacture.

Timeline of Vaccine Adjuvants Development



Some of Licensed Adjuvanted Vaccines for Human Use

Trade Name	Type	Adjuvant
CERVARIX	Human Papillomavirus vaccine (types 16, 18) (recombinant)	AS04 containing 3-O-desacyl-4’ -monophosphoryl lipid A (MPL) adsorbed on Aluminum hydroxide
FENDRIX	Hepatitis B vaccine (recombinant)	AS04 containing MPL adsorbed on Aluminum hydroxide
FLUAD	Inactivated influenza vaccine, surface antigen	MF59, squalene-based adjuvant
NUVAXOVID	COVID-19 Vaccine (recombinant)	Matrix-M containing Fraction-A and Fraction-C of Quillaja saponaria Molina extract
SHINGRIX	Herpes zoster vaccine (recombinant)	AS01B containing Quillaja saponaria Molina plant extract, fraction 21 (QS-21)
MOSQUIRIX	Plasmodium falciparum and hepatitis B vaccine (recombinant)	AS01E containing QS-21 and MPL

Yaohai's Adjuvants Manufacturing Capabilities

Under our GMP workshop with biosafety levels BSL-1 and BSL-2, Yaohai Bio-Pharma provides Contract Manufacturing Services for **microbial or plant-derived adjuvants, as well as recombinant cytokines as adjuvants.**

More specifically, we offer customized solutions for GMP grade **MPL, QS-21**, Recombinant Cytokines, and other vaccine adjuvants.

Equipments

- For microbial-derived adjuvants (such as MPL) or recombinant cytokines, large-scale stainless steel fermenters with sizes ranging from thousands to millions of litres can be combined with centrifugal, hollow fibre, and low-to-high pressure chromatography systems.

Fermentation System 2000 L

Disc Stack Centrifuge

- High pressure chromatography and a high-potency production suite may be appropriate for natural adjuvants such as QS-21.

High-potency Manufacturing Suite

High Pressure Chromatography

Microbial Biologics CRDMO

CONTACT US

Email: BD@yaohaibio.cn
www.yaohai-bio.com.cn

[CRO service]
www.yaohai-bio.com.cn/CRO_mRNA

[CDMO service]
www.yaohai-bio.com.cn/CDMO

[CMC development service platform]
www.yaohai-bio.com.cn/Platform_CMC

[Handbook]
www.yaohai-bio.com.cn/downloadfile

Facebook:
https://www.facebook.com/YaohaiBioPharma

Twitter:
https://twitter.com/YaohaiBiopharma

LinkedIn:
https://www.linkedin.com/company/YaohaiBio

Quora:
https://www.quora.com/profile/YaohaiBioPharma