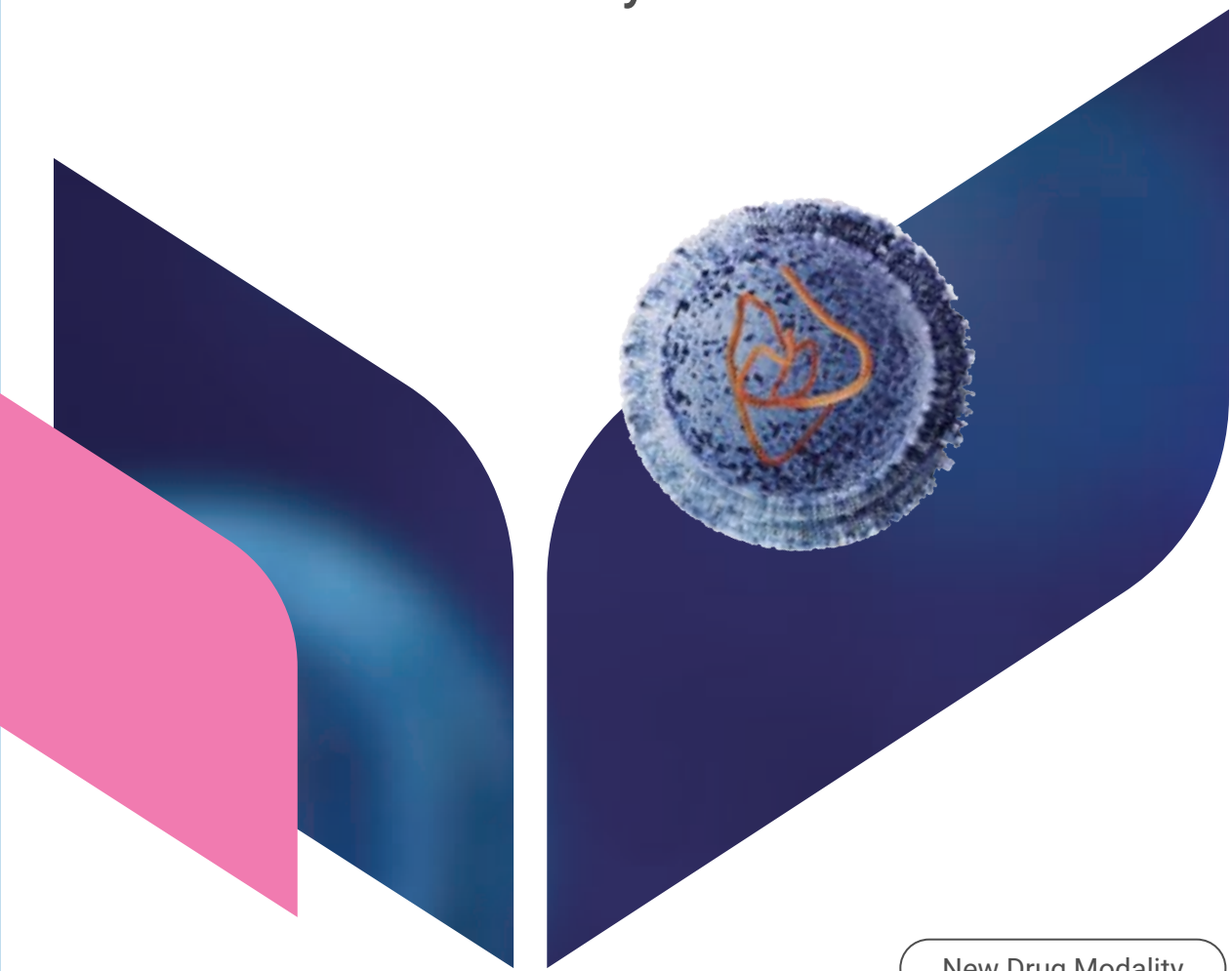
Laboratory Testing Division
Bioanalytical Services

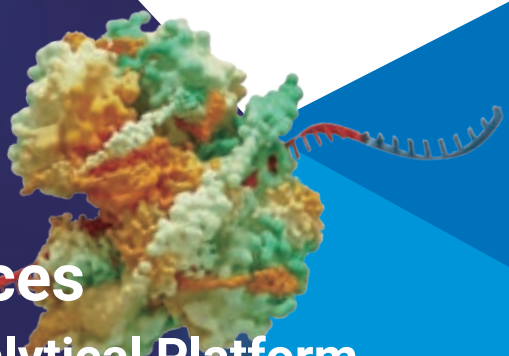
WuXi AppTec

mRNA-Based Therapeutics

Bioanalysis



New Drug Modality



Bioanalytical Services

Global Integrated Bioanalytical Platform

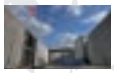
WuXi AppTec Bioanalytical Services operates globally and has leading integrated laboratories in China and the U.S. We adhere to GLP and GCP principles and strictly follow high industry regulatory standards. Our comprehensive platform, equipped with advanced instruments and staffed by experienced experts, provides professional one-stop services in preclinical/clinical mass spectrometry, immunochemistry, molecular/cellular, and central laboratory services to global clients.

Shanghai, China
Headquarters
Bioanalytical Services (Clinical)
Central Lab

Suzhou, China
Bioanalytical Services (Preclinical)
Antibody Service

New Jersey, USA
Bioanalytical Services (Preclinical/Clinical)
Central Lab

Chengdu, China
Bioanalytical Services (Preclinical)



Nantong, China
Bioanalytical Services (Preclinical)

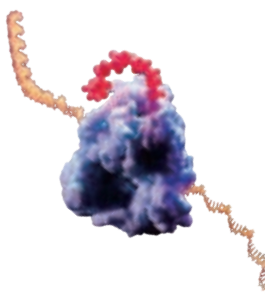
18+ Years lab operations in China and the U.S.
24,300 m² lab space

5,000+ small molecule validated methods
2,800+ large molecule validated methods
2M+ samples analyzed per year

Global Regulatory Standards

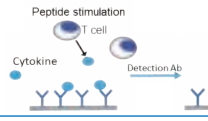
Successfully passed global regulatory inspections, comply with GLP, GCP, CAP quality standards
200+ China NMPA
30+ International regulatory agencies (U.S. FDA, OECD, EMA, PMDA, CAP)

Messenger RNA (mRNA), transcribed from DNA, serves as the template for protein synthesis. mRNA drugs often employ delivery systems like lipid nanoparticles (LNPs) and liposomes to enhance cellular uptake and protect the mRNA from degradation. These drugs have diverse therapeutic applications, including cancer vaccines, treatment of genetic diseases, and protein replacement therapies. Biopharmaceutical companies have been pushing the boundaries of mRNA therapy development in recent years.



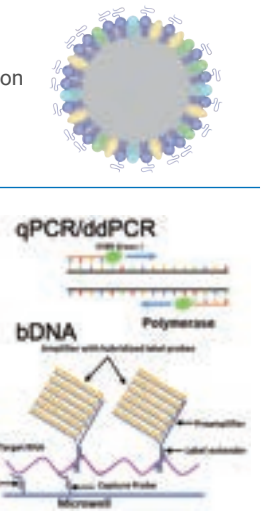
With over 20 years of expertise, WuXi AppTec's Bioanalytical Services (BAS) has extensive experience handling drug delivery systems such as LNPs and liposomes and supporting advanced therapy projects through the qPCR platform. We offer customized analytical strategies and comprehensive bioanalytical solutions tailored to the sponsor's needs and project specifics. We aim to expedite global IND, NDA, and BLA submissions, helping sponsors achieve their drug development milestones efficiently.

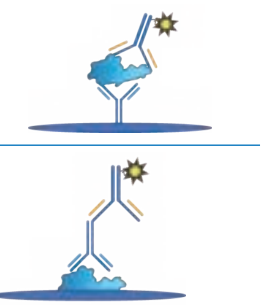
Bioanalytical Solutions for mRNA

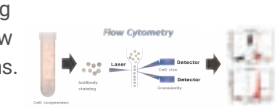
Detection Item		Analyte	Platform	Study Design
PK/TK/ Biodistribution	PK/TK/ Biodistribution	Cationic/ ionizable lipids, PEG-lipids	LCMS	▪ Protein precipitation or liquid-liquid extraction ▪ Multiple solvent options available for combination ▪ Establishment of a separate method for PEG-lipid detecti ▪ Use of longer gradient separation ▪ **Key point:** Selection of extraction solvent
		mRNA	qPCR/ddPCR /bDNA	▪ **qPCR:** As the starting material, RNA is transcribed into complementary DNA (cDNA) ▪ **bDNA**: Based on capture probes and "Z" probe specific complementary to the target molecule, the detection technology improves sensitivity through probe hybridization and signal amplification ▪ **Key points:** Primer design, capture/detection probe design
		Expressed protein	ELISA/MSD/ LCMS	**Critical reagents:** Expressed protein, anti-idiotype antibody
Immunogenicity	**ADA**	Anti-PEG Ab Anti-protein Ab	MSD/ELISA	▪ To detect anti-PEG or anti-expression product antibodies, the antibodies will be bound to the plate coated with the drug or the expression product ▪ **3 Tiers:** Screening, Confirmation, and Titer
	Cellular Immunology	Exogenous protein-specific immune cells	ELISpot	By culturing individual lymphocytes on a culture plate and th an antigen/peptide library, local production of cytokines can Enzyme-labeled antibodies can be used to detect the secret **Key points:** ▪ Antigen/peptide library ▪ Positive control ▪ Cell culture 
Pharmacodynamics/ Biomarkers	**IPT**	Immunophenotyping	FACS	▪ Immune cells can be classified and identified by detectin their surface markers, allowing for the assessment of hov drugs affect immune function and regulatory mechanism ▪ This approach is crucial for understanding the immunological impact of therapeutic interventions.
	Biomarker	Cytokines/ biomarkers	ELISA/MSD/ Luminex/ELLA/ Simoa/Gyros/ SMCxPRO	Biomarkers are essential tools for objectively monitoring and assessing biological and pathological processes and responses to pharmacological interventions. They are widel used to track clinical outcomes, pharmacodynamics (PD), and safety in drug development and clinical practice.

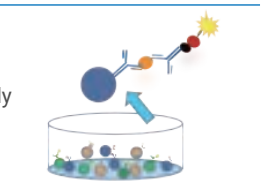
Bioanalytical Challenges and Experience with mRNA Drugs

Challenges	
Cationic/ Ionizable Lipids	▪ Poor water solubility ▪ Instability in biological matrix ▪ High protein binding ratio ▪ Difficulties in selecting internal standards ▪ Carryover
PEG-Lipid	▪ High interference and carryover ▪ Matrix effect ▪ Difficulties in selecting internal standards
mRNA	▪ RNA degradation ▪ Low tissue distribution with high background signals ▪ Primer specificity and amplification efficiency ▪ Consistency of extraction efficiency and pre-processing method in different types of samples
Anti-PEG Antibody **Anti-mRNA Antibody** **Anti-Protein Antibody**	▪ Low specificity and poor sensitivity of positive control ▪ Difficult to purify mRNA expression product ▪ Drug tolerance ▪ Anti-mRNA antibody: low immunogenicity, difficult to prepare positive control, low specificity and poor sensitivity
Expressed Protein	▪ Distinguish endogenous protein, poor sensitivity ▪ Low expression or absence of target proteins ▪ Significant endogenous protein interference ▪ Difficulties in purifying mRNA expression product ▪ Stability of real biological samples and the complexity of obtaining samples
Neutralizing Antibody	▪ Drug tolerance ▪ Robustness ▪ Sensitivity
Biomarker	▪ Robustness ▪ Sensitivity ▪ Multiple cytokines









mRNA Drug Case Study

Background:

Lipid nanoparticle-mRNA (LNP-mRNA) drugs are formulated to enhance the stability and delivery of modified mRNA by encapsulating it within lipid nanoparticles (LNPs). This encapsulation enables the expression of specific proteins. The adaptability of these drugs allows for customization with various mRNA sequences, enabling the targeted expression and regulation of proteins. This approach holds significant promise in fields such as oncology, infectious disease prevention and treatment, vaccine development, and rare disease therapies. The following case study presents an analytical strategy for the preclinical safety evaluation of LNP-based mRNA advanced therapy drugs.

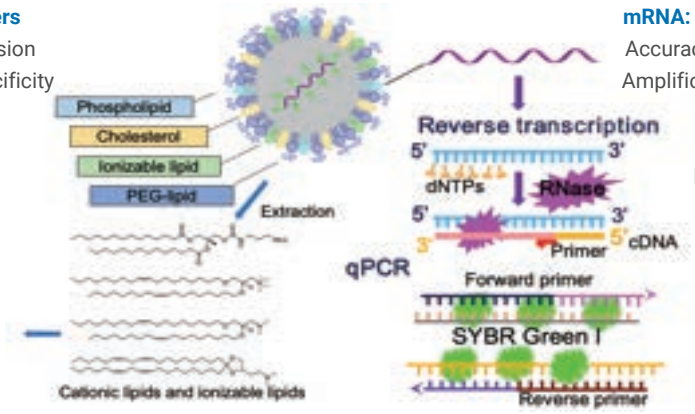
Study Design:

We offer tailored services to support IND submissions in alignment with global regulatory guidelines. Our comprehensive approach integrates multiple platforms, including LC-MS/MS, ELISA, qPCR, FACS, and Luminex, to facilitate the preclinical safety evaluation of LNP-mRNA drugs, particularly in the treatment of rare diseases.

LNP, mRNA, Immunogenicity, Expressed Protein Bioanalysis Strategy

LNP : MV Parameters

Accuracy and precision
Selectivity and specificity
Extraction recovery
Matrix effect
Dilution linearity
Stability
Carryover

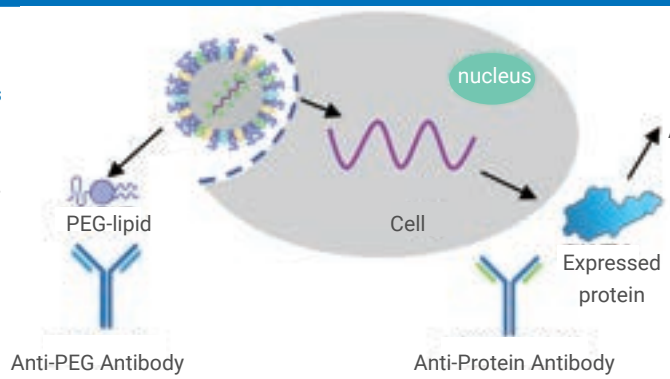


mRNA: MV Parameters

Accuracy and precision
Amplification efficiency
Assay range
Matrix effect
Dilution linearity
Hook effect
Stability
Parallelism

ADA:
MV Parameters

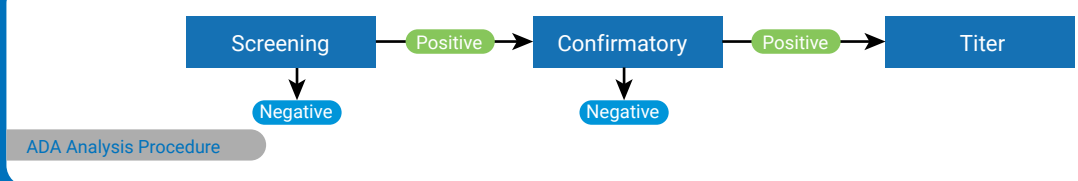
Precision
Sensitivity
Cut-point factor
Matrix effect
Stability
Hook effect
Drug tolerance
Robustness



Expressed Protein:
MV Parameters

Accuracy and precision
Assay range
Matrix effect
Dilution linearity
Hook effect
Stability
Parallelism

Immunogenicity Bioanalysis Strategy



Our Advantages

Global Integrated Platform: We provide one-stop services to global sponsors, from preclinical to clinical stages, supported by multiple BAS sites worldwide.

Customized Services: Our sponsor-centric professional project management team delivers tailored services to meet your specific needs.

Experienced Team: Our core technical team members boast over 10 years of experience, with our management team having more than 20 years.

Statistical Services: Our immunogenicity cut-point determination offers expedited data delivery, ensuring you quickly get accurate, reliable results. Our services are grounded in scientific rigor, providing enhanced accuracy and dependable outcomes.

With extensive experience from more than 20 mRNA-based therapeutics projects using a qPCR platform, including AAVx, LNP, and exosome-based delivery systems, we are dedicated to accelerating your projects and addressing a wide range of analytical challenges.

Common Industry References

1. Method Validation for Quantitation in Biological Samples, Chinese Pharmacopoeia (2020)
2. Bioanalytical Method Validation and Study Sample Analysis M10, International Council for Harmonisation
3. Bioanalytical Method Validation, U.S. Food and Drug Administration
4. Immunogenicity Testing of Therapeutic Protein Products—Developing and Validating Assays for Anti-Drug Antibody Detection, U.S. Food and Drug Administration

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