



## Analytical Support for mRNA Therapeutics

Thorough analytical testing is essential to verify the identity, purity, and stability of mRNA vaccines and therapeutics. Leveraging our extensive experience supporting leading mRNA COVID-19 vaccine manufacturers with raw material, drug substance, and drug product release, Avance Biosciences is prepared to assist you in ensuring the quality of your mRNA therapeutics.

### mRNA DS/DP Testing (CGMP)

Critical Quality Attributes	Assay	Description
Identity	mRNA sequence identity confirmation	Confirm the sequence of mRNA by Sanger Sequencing or NGS or RT-qPCR
	Identity of RNA mixture	Confirm the presence of multi-valent mRNA drug substance or product using RT-qPCR or ddPCR
Content	RNA concentration	Quantify mRNA using UV Spectroscopy, RT-qPCR, or ddPCR
	RNA encapsulation efficiency	Determine mRNA/LNP encapsulation efficiency using fluorescence-based assay
	RNA Ratio Determination	Confirm the ratio of the mRNAs in multi-valent mRNA vaccine drug substance or product using RT-qPCR or ddPCR
Integrity	RNA Size and Integrity	Determine mRNA intactness using CE or Agarose gel electrophoresis
Purity	Product related impurities - dsRNA	Detect various dsRNAs using immunoblotting, native and denaturing gel electrophoresis, or ELISA
	Process related impurities-residual DNA template	qPCR-based assay to detect potential DNA contamination

## mRNA DS/DP Testing (CGMP)

Critical Quality Attributes	Assay	Description
<b>Purity</b>	Process related impurities -residual T7 RNA polymerase content	ELISA-based assay to detect potential DNA contamination
<b>Potency</b>	Expression of target protein	Develop and validate a custom cell-based assay to determine potency
<b>Safety</b>	Endotoxin	USP <85>
	Bioburden	USP <61>, <62>
	Sterility	USP <71>
<b>Other</b>	Appearance	USP <790>
	pH	USP <791>
	Osmolality	USP <785>

## Preclinical/Clinical Testing (GLP or non-GLP)

Tests	Description
<b>mRNA/LNP Biodistribution Study</b>	Develop and validate RT-qPCR and/or ddPCR assays and test tissues/blood from various animal models under GLP or non-GLP.
<b>Pharmacokinetics (PK) Study</b>	Develop and validate RT-qPCR and/or ddPCR assays and test human bodily fluids for mRNA expression.
<b>Pharmacodynamics (PD) Study</b>	Develop and validate custom assays to measure biomarker levels or protein activity using flow cytometry, Western blot, ELISA, or MSD.
<b>Anti-Drug Antibody (ADA) Analysis</b>	Develop and validate ADA assays by employing multi-tiered testing approach using ELISA, MSD, or Flow Cytometry.
<b>Cytokine Release and Immune Profiling</b>	Develop and validate custom assays to assess the immune response to the mRNA therapeutic using ELISA, MSD, or Flow Cytometry.