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3.2.S.2.3. MATERIALS USED IN MANUFACTURE [BNT, MAINZ, AND RENTSCHLER]

A list of the materials used in the manufacture of BNT162b2 drug substance is given below. The site of manufacture has a vendor management program, including appropriate quality systems, to ensure control of raw materials used for GMP manufacturing. As a result, these materials are purchased from approved suppliers. The raw materials used in the drug substance manufacturing process are tested and released upon receipt in accordance with internal raw material specifications. Specifications for all non-compendial grade raw materials are described in [Section 3.2.S.2.3.2](#).

Purified water or water for injection (WFI) manufactured at the facility or purchased from qualified suppliers is used throughout the drug substance process and meets USP/Ph. Eur. requirements.

3.2.S.2.3.1. Materials Used in the Manufacturing Processes

3.2.S.2.3.1.1. Solutions and Buffers Used in the Manufacturing Processes

Table 3.2.S.2.3-1 outlines the solutions and buffers used during the commercial scale in vitro transcription (IVT), DNase I digestion, proteinase K digestion, ultrafiltration/diafiltration (UFDF), and final filtration and dispense processes.

Table 3.2.S.2.3-1. Solutions and Buffers Used in the Manufacturing Processes

Use	Process Step(s)	Composition
10X transcription buffer	IVT	4.2 1st ind
Calcium chloride solution	DNase I digestion	50 mM calcium chloride
EDTA solution	DNase I digestion	500 mM EDTA
4.2 1st ind dilution buffer	UFDF	4.2 1st ind
Diafiltration 1 buffer	UFDF	4.2 1st ind
Formulation buffer	UFDF, Final filtration and dispense	4.2 1st ind

Abbreviations: 4.2 1st ind

EDTA = edetate disodium dihydrate

3.2.S.2.3.1.2. Raw Materials Used in the Manufacturing Processes

[Table 3.2.S.2.3-2](#) provides the list of raw materials used during the manufacturing processes. Raw materials as listed in the table below are used to prepare the listed solution/buffers.

Table 3.2.S.2.3-2. Raw Materials Used in the Manufacturing Processes

Raw Material	Grade	Solution/Buffer
5'-cap solution ^a	Non-compendial	100 mM 5'-cap
4.2 1st ind	Ph. Eur. / USP-NF	4.2 1st ind
ATP solution ^a	Non-compendial	100 mM adenosine 5'-triphosphate
Calcium chloride dihydrate	Ph. Eur.	50 mM calcium chloride
CTP solution ^a	Non-compendial	100 mM cytidine 5'-triphosphate
DNase I ^b	Non-compendial	DNase I
EDTA	Ph. Eur. / USP-NF	500 mM EDTA, 4.2 1st ind
GTP solution ^a	Non-compendial	100 mM guanosine 5'-triphosphate
HEPES	Non-compendial	4.2 1st ind
Hydrochloric acid	Ph. Eur.	500 mM EDTA
N1-methylpseudo UTP solution ^a	Non-compendial	100 mM N1-methylpseudouridine 5'-triphosphate
Proteinase K ^b	Non-compendial	Proteinase K
Pyrophosphatase ^b	Non-compendial	Pyrophosphatase
RNase inhibitor ^b	Non-compendial	RNase inhibitor
Sodium hydroxide	Ph. Eur. / USP-NF	500 mM EDTA, 4.2 1st ind
T7 polymerase ^b	Non-compendial	T7 RNA polymerase
10X transcription buffer	Non-compendial	4.2 1st ind

a. Starting material

b. 50% glycerol in buffer solution

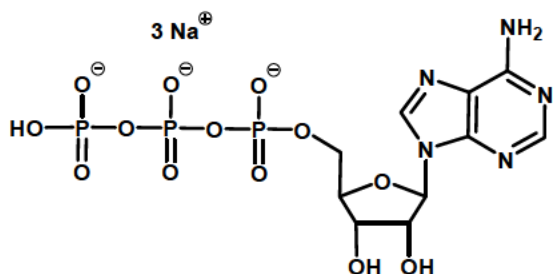
Abbreviations: HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; 4.2 1st ind :
 EDTA = edetate disodium dihydrate; Ph. Eur. = European Pharmacopoeia; NF = National Formulary; USP =
 United States Pharmacopeia

3.2.S.2.3.2. Control of Non-compendial Starting Materials and Raw Materials

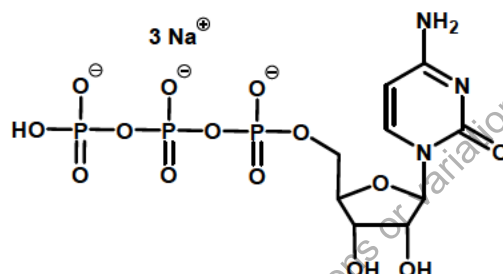
3.2.S.2.3.2.1. Starting Materials

Starting materials are defined as a component, reagent or material used during the manufacture of an mRNA vaccine product that is intended to be part of the final product. The following starting materials are purchased as solutions: ATP solution, CTP solution, GTP solution, N1-methylpseudo UTP solution, and 5'-cap solution, and [Figure 3.2.S.2.3-1](#) provides the structures of these starting materials. The definition of starting material was also extended to linear DNA template, which is not part of the final product, but defines the sequence of the RNA product (see [Section 3.2.S.2.3 Source, History and Generation of Plasmids](#)).

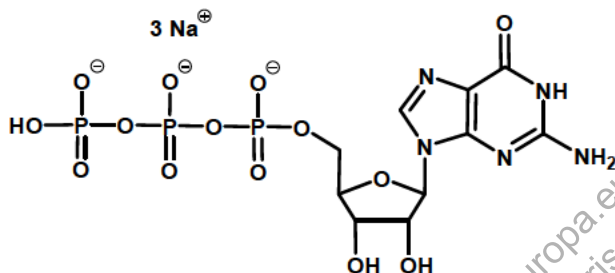
Figure 3.2.S.2.3-1. Structures of ATP, CTP, GTP, N1-methylpseudo UTP, and 5'-cap



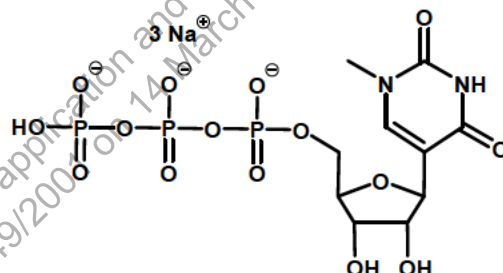
ATP



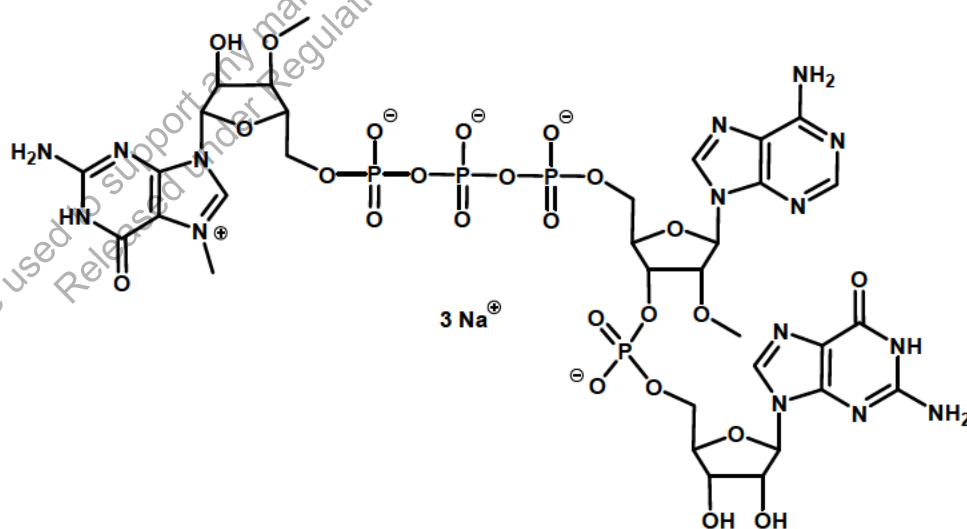
CTP



GTP



N1-methylpseudo UTP



5'-cap

3.2.S.2.3.2.2. Acceptance Criteria for Non-compendial Starting Materials and Raw Materials used in Manufacturing

The current acceptance criteria for non-compendial starting materials and raw materials used during IVT, DNase I digestion, proteinase K digestion, and UFDF processes are presented in [Table 3.2.S.2.3-3](#). The characteristics tested by or on behalf of the drug substance manufacturer prior to use of the material in manufacture of the drug substance are indicated. The remaining characteristics are listed on the vendor's certificate of analysis and are required to release the material for use in the manufacture of drug substance. The acceptance criteria for linear DNA template are shown in [Section 3.2.S.2.3 Source, History and Generation of Plasmids](#).

Table 3.2.S.2.3-3. Acceptance Criteria for Non-Compendial Starting Materials and Raw Materials Used in Manufacturing

Material	Characteristic	Acceptance Criteria
5'-cap solution ^a	Identity ^b	Identity confirmed
	Purity	4.2 1st ind
	Concentration	4.2 1st ind
ATP solution ^a	Identity ^b	Identity confirmed
	Purity	4.2 1st ind
	Concentration	4.2 1st ind
CTP solution ^a	Identity ^b	Identity confirmed
	Purity	4.2 1st ind
	Concentration	4.2 1st ind
DNase I ^c	Identity ^b	Identity confirmed
	Activity	4.2 1st ind
	Purity	4.2 1st ind
GTP solution ^a	Identity ^b	Identity confirmed
	Purity	4.2 1st ind
	Concentration	4.2 1st ind
HEPES	Appearance ^b	White crystals or crystalline powder (Passes test)
	Identity ^b	Passes test
N1-methylpseudo UTP solution ^a	Identity ^b	Identity confirmed
	Purity	4.2 1st ind
	Concentration	4.2 1st ind
Proteinase K ^c	Identity ^b	Identity confirmed
	Activity	4.2 1st ind
Pyrophosphatase ^c	Identity ^b	Identity confirmed
	Activity	4.2 1st ind
	Purity	4.2 1st ind
RNase inhibitor ^c	Identity ^b	Identity confirmed
	Activity	4.2 1st ind
	Purity	4.2 1st ind
T7 polymerase ^c	Identity ^b	Identity confirmed
	Activity	4.2 1st ind
	Purity	4.2 1st ind
10X transcription buffer	Identity ^b	Identity confirmed
	Content HEPES	4.2 1st ind
	Content Mg(OAc) ₂ *4 H ₂ O	4.2 1st ind

- a. Starting material
b. Internal assay
c. 50% glycerol in buffer solution

The ID testing listed above, along with inspection of materials received and examination of vendor certificate of analysis, will be performed on all batches. Additional material testing will be performed and provided when available (See [Module 2.3 Introduction to Quality Overall Summary](#)).

3.2.S.2.3.3. Filter Materials Used in the Drug Substance Manufacturing Process

The filter materials have been shown to be suitable for use in drug substance manufacturing. In-use testing at laboratory and pilot scale did not reveal any detrimental effects on drug substance quality or significant recovery losses. Table 3.2.S.2.3-4 lists the filter materials by process stage used during the manufacture of the drug substance.

Cellulose acetate is the material of construction for the process-related 0.45/0.2 µm dual-layer filters. Stabilized cellulose is the material of construction for ultrafiltration/diafiltration (UFDF) membranes used for buffer exchange.

Table 3.2.S.2.3-4. Filter Materials Used in the Manufacture of Drug Substance

Process Stage	Filter type / application	Filter Materials
UFDF	UF membrane filter 300 kDa MWCO	Stabilized cellulose
Final filtration	Dual-layer membrane filter 0.45/0.2 µm / drug substance filtration	Cellulose acetate
Abbreviations: MWCO = Molecular weight cut-off; UFDF = ultrafiltration/diafiltration		

3.2.S.2.3.4. Control of Source and Starting Materials of Biological Origin

[Table 3.2.S.2.3-5](#) provides a summary of materials of biological origin used in manufacturing. Raw material vendors are qualified for sourcing materials for use in the manufacturing process. All materials used in the manufacture of drug substance have been investigated for the origin of animal material and have been determined to comply with “Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMA/410/01).” Additional information on raw material biological origin is in [Section 3.2.A.2 Adventitious Agents Safety Evaluation](#).

Table 3.2.S.2.3-5. Materials of Animal Origin Used in Drug Substance Manufacturing

Raw Material	Manufacturing Process Stage (Use)
Proteinase K	Drug substance manufacturing (Proteinase K digestion step)
Filters of various sizes	Drug substance manufacturing
Flexible containers (Bag systems) used at various processing steps and to hold final drug substance	Drug substance manufacturing
Clear C-flex tubing, various sizes, including manifold assemblies provided by vendors	Drug substance manufacturing
Tubing assembly	Drug substance manufacturing