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### 3.2.S.2.2. MANUFACTURING PROCESS [BNT MAINZ AND RENTSCHLER]

#### 3.2.S.2.2.1. Overview of Manufacturing Process

This section includes the description of the manufacturing process for BNT162b2 drug substance. The RNA is first synthesized via an in vitro transcription (IVT) followed by DNase I and proteinase K digestion steps, which aid in purification. The crude RNA is then purified through a 2-stage ultrafiltration/diafiltration (UFDF). Lastly, the RNA undergoes a final filtration before being dispensed and stored frozen.

A flow diagram for the drug substance process is shown in [Table 3.2.S.2.2-1](#). For each process step, this flow diagram lists the process inputs (materials added) and the process controls (process parameters, material attributes, process performance attributes (PPA), in-process tests for control (IPT-C) and in-process tests for monitoring (IPT-M)).

IPT-Cs are in-process tests used to control a QA/CQA within a specified range so that it meets the desired drug substance/drug product quality. The IPT-Cs have an associated acceptance criterion. These IPT-Cs are tabulated in this section with their associated acceptance criterion and also described in [Section 3.2.S.2.4 In Process Test Methods \[BNT Mainz and Rentschler\]](#).

In addition to IPT-Cs, in-process tests for monitoring (IPT-M) have been implemented throughout the process to ensure consistency of the manufacturing process ([Section 3.2.S.2.6 Control Strategy](#)). IPT-Ms are in-process tests used to monitor a QA/CQA to either ensure that it is consistent with respect to previous process history or for forward processing. The monitoring tests may have action limits. These IPT-Ms are described in [Section 3.2.S.2.4 In-Process Test Methods \[BNT Mainz and Rentschler\]](#).

All process parameters are defined and controlled within the applicable ranges detailed in the batch records and standard operating procedures. Characterization of each of the unit operations, to date, and the justification of acceptable ranges for the process parameters are described in [Section 3.2.S.2.6 Process Development and Characterization](#).

All unit operations are performed at ambient temperature (15-25 °C), unless otherwise stated.

BNT162b2

## 3.2.S.2.2 Description of Manufacturing Process and Process Controls

Manufacturing Process [BNT Mainz and Rentschler]

**Table 3.2.S.2.2-1. RNA Manufacturing Process**

Step #	Process Inputs	Process Step	Process Controls
1	ATP solution, CTP solution, N1-methylpseudo UTP solution, GTP solution, 5'-cap solution, RNase inhibitor, 10X transcription buffer, Linear DNA Template, Pyrophosphatase, T7 polymerase, Water for Injection	In Vitro Transcription (IVT)	4.2 1st ind.
2	Calcium chloride solution, DNase I, EDTA	DNase I Digestion	
3	Proteinase K	Proteinase K Digestion	
4	4.2 1st ind. dilution buffer, diafiltration I buffer, formulation buffer	Proteinase K pool transported to Rentschler	
		Ultrafiltration/Diafiltration (UFDF) recovered into 1 <sup>st</sup> flexible container	
		Product Dilution	
5		UFDF pool	
		Final 0.45/0.2 µm filtration into 2 <sup>nd</sup> flexible container	
		Drug Substance	
		Dispense	
		Drug Substance in EVA Flexible Containers (FCs)	

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**3.2.S.2.2.2. In Vitro Transcription (IVT)**

The primary objective of the IVT step is synthesis of RNA for drug substance production.

To begin the IVT step, individual components are thawed and added to the reaction vessel, including ATP solution (100 mM adenosine 5'-triphosphate), CTP solution (100 mM cytidine 5'-triphosphate), N1-methylpseudo UTP solution (100 mM N1-methylpseudouridine 5'-triphosphate), GTP solution (100 mM guanosine 5'-triphosphate), 5'-cap solution (100 mM 5'-cap), and water for injection (WFI). RNase inhibitor, 10X transcription buffer

**4.2 1st ind.**

and the linear DNA template are added to the reaction vessel. During the reagent additions, pre-enzyme agitation and temperature control are initiated at target ranges. Finally, pyrophosphatase and T7 polymerase (T7 RNA polymerase) are added to the reaction vessel and agitation is increased to the post-enzyme agitation rate. The above volume additions total to the IVT starting target volume **4.2 1st ind.** After these enzyme additions, the incubation time during GTP/N1-methylpseudo UTP bolus feeds begins. The parameter ranges are shown in Table 3.2.S.2.2-2.

**4.2 1st ind.**

Upon completion of the final IVT incubation time, the process immediately proceeds to the DNase I digestion operation.

The IVT step is controlled using the process parameters shown in Table 3.2.S.2.2-2.

**Table 3.2.S.2.2-2. In Vitro Transcription Process Parameters**

Parameter	Acceptable Range
<b>4.2 1st ind.</b>	

**3.2.S.2.2.3. DNase I Digestion**

The primary objective of the DNase I digestion step is to reduce the size of linear DNA template and enable subsequent removal across the ultrafiltration/diafiltration step.

A calcium chloride solution (50 mM calcium chloride) and a DNase I solution are added at the end of the final IVT incubation. 4.2 1st ind.

Upon completion of the DNase I incubation time, EDTA (500 mM EDTA) is added 4.2 1st ind. After the EDTA addition, the process proceeds with the proteinase K digestion.

The DNase I digestion step is controlled using the process parameters shown in Table 3.2.S.2.2-3.

**Table 3.2.S.2.2-3. DNase I Digestion Process Parameters**

Parameter	Acceptable Range
4.2 1st ind.	

#### 3.2.S.2.2.4. Proteinase K Digestion

The primary objective of the proteinase K digestion step is to reduce the size of proteins in the reaction mixture for subsequent removal across the ultrafiltration/diafiltration step.

Proteinase K solution is added to the reaction vessel and incubated for a predetermined amount of time. 4.2 1st ind. At the completion of proteinase K incubation time, the pool is filled into 4.2 1st ind. bags, labeled and packed into corresponding shell and shipper.

4.2 1st ind.

The proteinase K digestion step is controlled using the process parameters shown in Table 3.2.S.2.2-4.

**Table 3.2.S.2.2-4. Proteinase K Digestion Parameters**

Parameter	Acceptable Range
4.2 1st ind.	

Sanitary control IPT-Ms and their action limits are provided in Table 3.2.S.2.2-5.

**Table 3.2.S.2.2-5. In-Process Tests (Monitoring) for Proteinase K Sanitary Control**

Sample	Test	Action Limit
4.2 1st ind.	Bioburden (CFU/10mL)	4.2 1st ind.
	Endotoxin (EU/mL)	
	Bioburden (CFU/10mL)	
	Endotoxin (EU/mL)	

**3.2.S.2.2.5. Ultrafiltration/Diafiltration (UFDF)**

The UFDF step reduces small process-related impurities and concentrates and buffer exchanges the RNA into the final DS formulation (4.2 1st ind.

To prepare for the UFDF step, the UFDF membranes (4.2 1st ind.) are rinsed with WFI then equilibrated with diafiltration 1 buffer (4.2 1st ind.

The pH and conductivity of the equilibrated membranes are verified.

Prior to UFDF, the post-proteinase K pool is diluted (4.2 1st ind.) with an (4.2 1st ind.) dilution buffer (4.2 1st ind.

The diluted proteinase K pool then undergoes a 2-stage diafiltration: (4.2 1st ind.

Based on the retentate RNA concentration determined after diafiltration 2, the diafiltered retentate is then concentrated, if needed, and recovered into a flexible container. Formulation buffer may be added (4.2 1st ind.). An in-process test for control (IPT-C), with established acceptance criteria, is then performed for RNA concentration (as described in [Section 3.2.S.2.4 Manufacturing Process](#)). After use, the UFDF membranes are cleaned with 1 N sodium hydroxide solution and stored in 0.1 N sodium hydroxide solution.

The UFDF is controlled using the following process parameters shown in Table 3.2.S.2.2-6.

**Table 3.2.S.2.2-6. UFDF and Formulation Process Parameters**

Parameter	Acceptable Range
4.2 1st ind.	

The UFDF step in-process tests for control (IPT-C) is shown in Table 3.2.S.2.2-7.

**Table 3.2.S.2.2-7. In-Process Tests (Control) for UFDF**

Test	Acceptance Criteria
RNA concentration (mg/mL)	4.2 1st ind.



Sanitary control IPT-Ms and their action limits are provided in Table 3.2.S.2.2-8.

**Table 3.2.S.2.2-8. In-Process Tests (Monitoring) for UFDF Sanitary Control**

Sample	Test	Action Limit
4.2 1st ind.	Bioburden (CFU/10mL)	4.2 1st ind.
	Endotoxin (EU/mL)	

### 3.2.S.2.2.5.1. UFDF Membrane Life Validation

The UFDF membrane lifetime will be established through at-scale concurrent validation studies that are currently ongoing ([Section 3.2.S.2.5 Additional Process Evaluation \[BNT Mainz and Rentschler\]](#)).

### 3.2.S.2.2.6. Final Filtration and Dispense

The UFDF pool undergoes a bulk final 0.45/0.2 µm filtration into a flexible container. Final drug substance release testing is performed at this stage. The drug substance (DS) is then dispensed into ethylene vinyl acetate (EVA) flexible containers (FC) ([Section 3.2.S.6 Container Closure](#), note the terms “flexible container” (FC) or “bag” are equivalent in describing the EVA containers).

The DS pool can be maintained at ambient temperature for less than 24 hours after final filtration and prior to start of drug substance dispensing.

Sanitary control IPT-Ms and their action limits are provided in Table 3.2.S.2.2-9.

**Table 3.2.S.2.2-9. In-Process Tests (Monitoring) for pre-filtration Sanitary Control**

Sample	Test	Action Limit
4.2 1st ind.	Bioburden (CFU/10mL)	4.2 1st ind.
	Endotoxin (EU/mL)	

### 3.2.S.2.2.7. Drug Substance Storage

The DS FCs are frozen and stored at -25 °C to -15 °C. See [Section 3.2.S.7.1 Stability Summary and Conclusions](#) for stability information.

### 3.2.S.2.2.8. Transportation

DS FCs shipments using an insulated shipper are qualified for a shipping time of up to 89 hours at temperatures of -25 to -15 °C as supported in [Section 3.2.S.2.5 Shipping Performance Qualification \[BNT Mainz and Rentschler\]](#).

**3.2.S.2.2.9. Bulk Final Refiltration Procedure**

In the event that the post-use integrity test on the final 0.45/0.2 µm filter fails or pinholes in flexible bags or leaks in transfer tubings occur, the bulk DS may be refiltered through an unused 0.45/0.2 µm filter. The Bulk Filtration and Dispense Refiltration step is performed in the same manner as the initial Final Filtration and Dispense step. The bulk DS is mixed and sampled for bioburden and endotoxin evaluation prior to being filtered through the new 0.45/0.2 µm filter. The newly used 0.45/0.2 µm filter is flushed with WFI and integrity tested. The results of a refiltration integrity test must pass or the batch is rejected. Supportive information is provided in [Section 3.2.S.2.5 Manufacturing Process \[BNT Mainz and Rentschler\]](#).