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3.2.S.2.2. MANUFACTURING PROCESS [ANDOVER]

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) step 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 [Figure 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)).

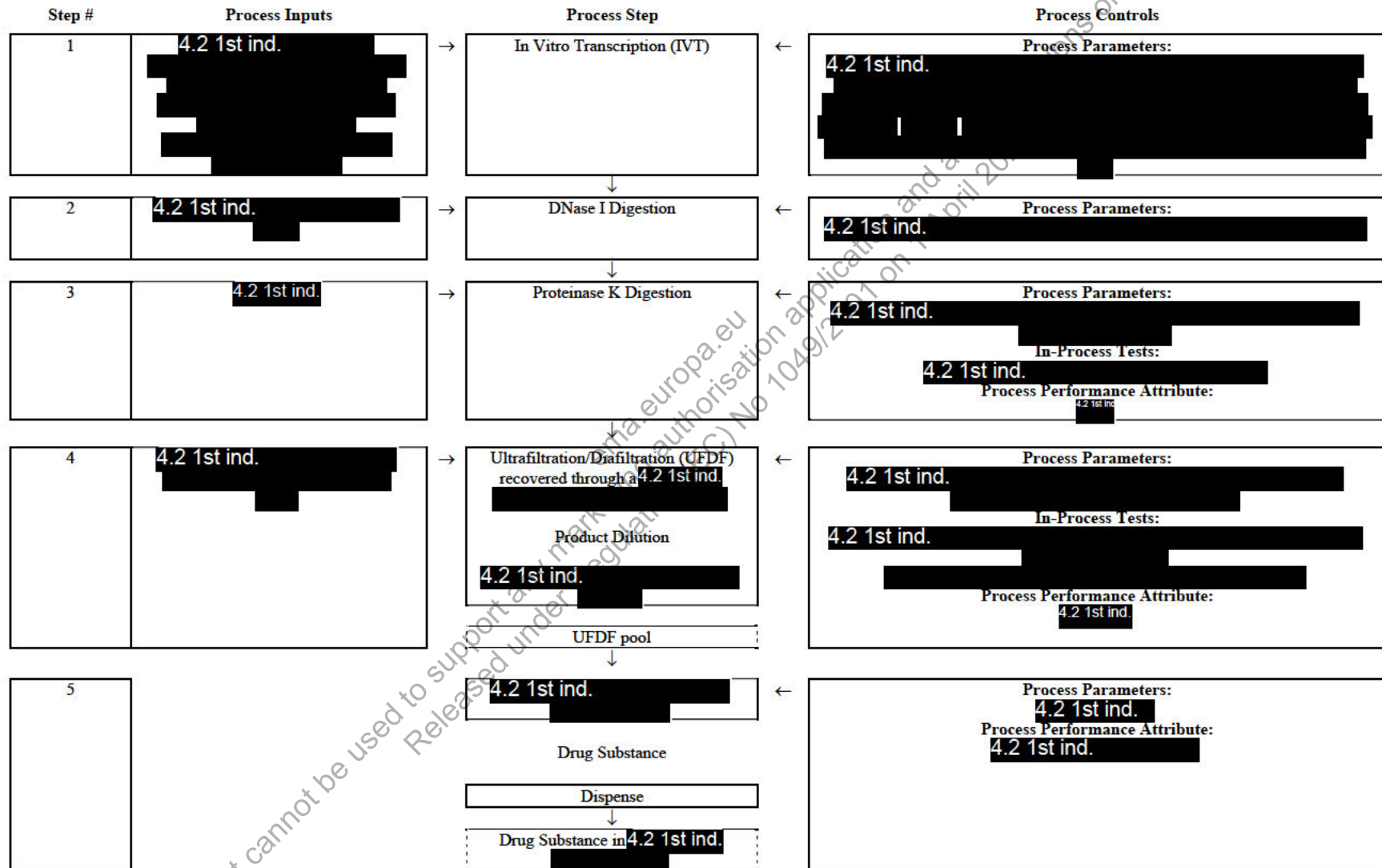
IPT-Cs are in-process tests used to control a QA/CQA within a specified range so that it meets the desired drug substance/drug product quality. The IPT-Cs have an associated acceptance criterion. These IPT-Cs are tabulated in this section with their associated acceptance criterion and also described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

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 \[Andover\]](#).

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 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.

Figure 3.2.S.2.2-1. RNA Manufacturing Process



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

The parameter ranges can be seen in Table 3.2.S.2.2-1.

The IVT step is controlled using the process parameters shown in Table 3.2.S.2.2-1.

Table 3.2.S.2.2-1. In Vitro Transcription Process Parameters

[illegible]

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

4.2 1st ind

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

Table 3.2.S.2.2-2. 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.

4.2 1st ind

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

Table 3.2.S.2.2-3. 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.2.2-4](#).

Table 3.2.S.2.2-4. 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)	4.2 1st ind

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

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

4.2 1st ind

The UFDF is controlled using the following process parameters shown in Table 3.2.S.2.2-5.

Table 3.2.S.2.2-5. UFDF 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.2.2-6.

Table 3.2.S.2.2-6. 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.2.2-7.

Table 3.2.S.2.2-7. 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)	4.2 1st ind
4.2 1st ind	Bioburden (CFU/10mL)	4.2 1st ind
	Endotoxin (EU/mL)	4.2 1st ind

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 \[Andover\]](#)).

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 FCs can be held at 2 – 8 °C for up to 72 hours prior to freezing ([Section 3.2.S.2.5 Hold Times \[Andover\]](#)).

3.2.S.2.2.7. Drug Substance Storage

The DS FCs are frozen and stored between -15 °C and -25 °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 106 hours at temperatures ≤ -15 °C as supported in [Section 3.2.S.2.5 Shipping Performance Qualification \[Andover\]](#).

3.2.S.2.2.9. Bulk Final Refiltration Procedure

4.2 1st ind.

