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3.2.P.3.3. LIPID NANOPARTICLE (LNP) PRODUCTION AND BULK DRUG PRODUCT FORMULATION – BIVALENT – [BNT MARBURG]

3.2.P.3.3.1. Flow Diagram

The process flow diagram for the BNT162b2 Bivalent LNP production and bulk drug product formulation manufacturing process is presented in Figure 3.2.P.3.3-1.

Figure 3.2.P.3.3-1. Flow Diagram of the Bivalent Drug Product Manufacturing Process and Process Controls

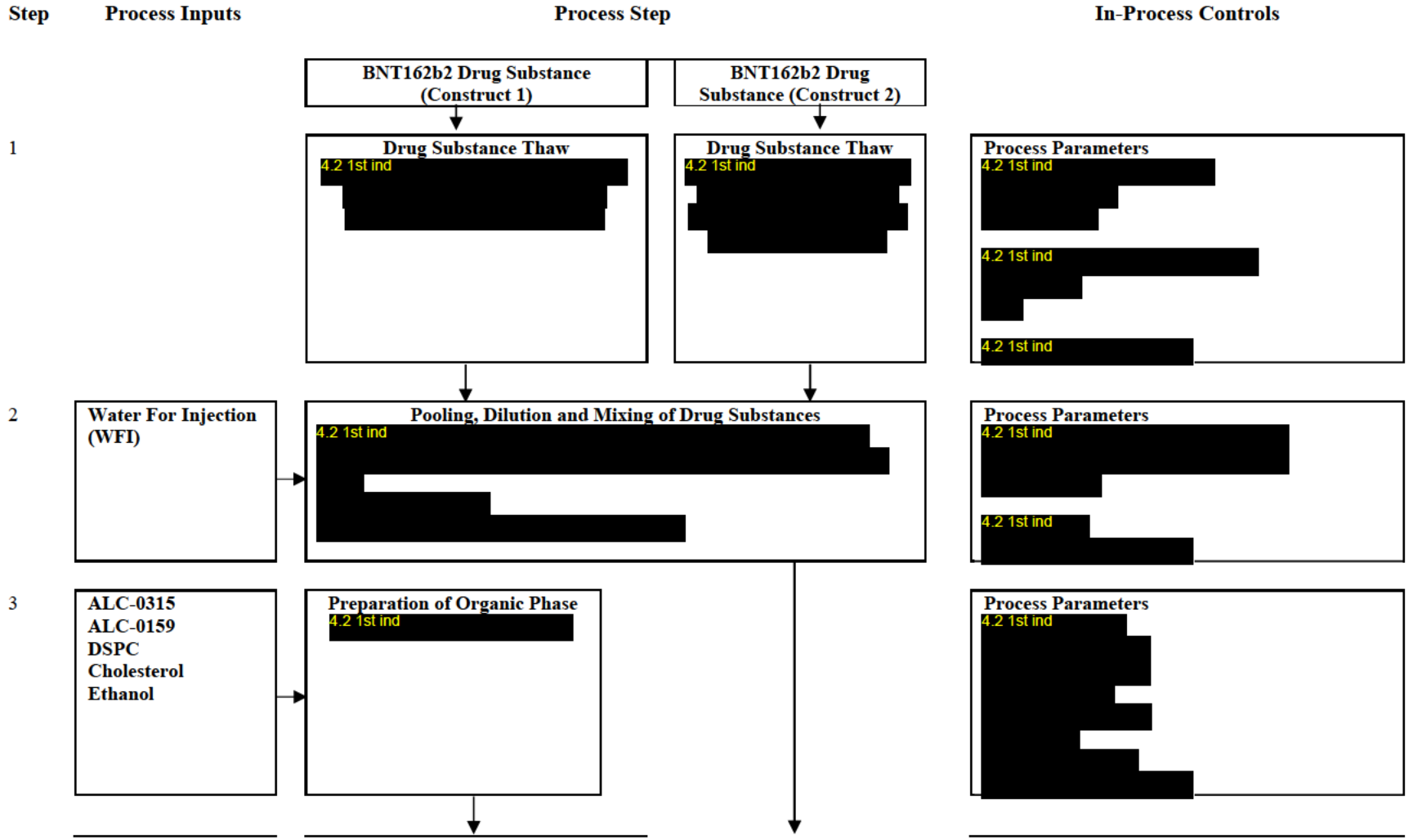

Figure 3.2.P.3.3-1. Flow Diagram of the Bivalent Drug Product Manufacturing Process and Process Controls

Figure 3.2.P.3.3-1. Flow Diagram of the Bivalent Drug Product Manufacturing Process and Process Controls

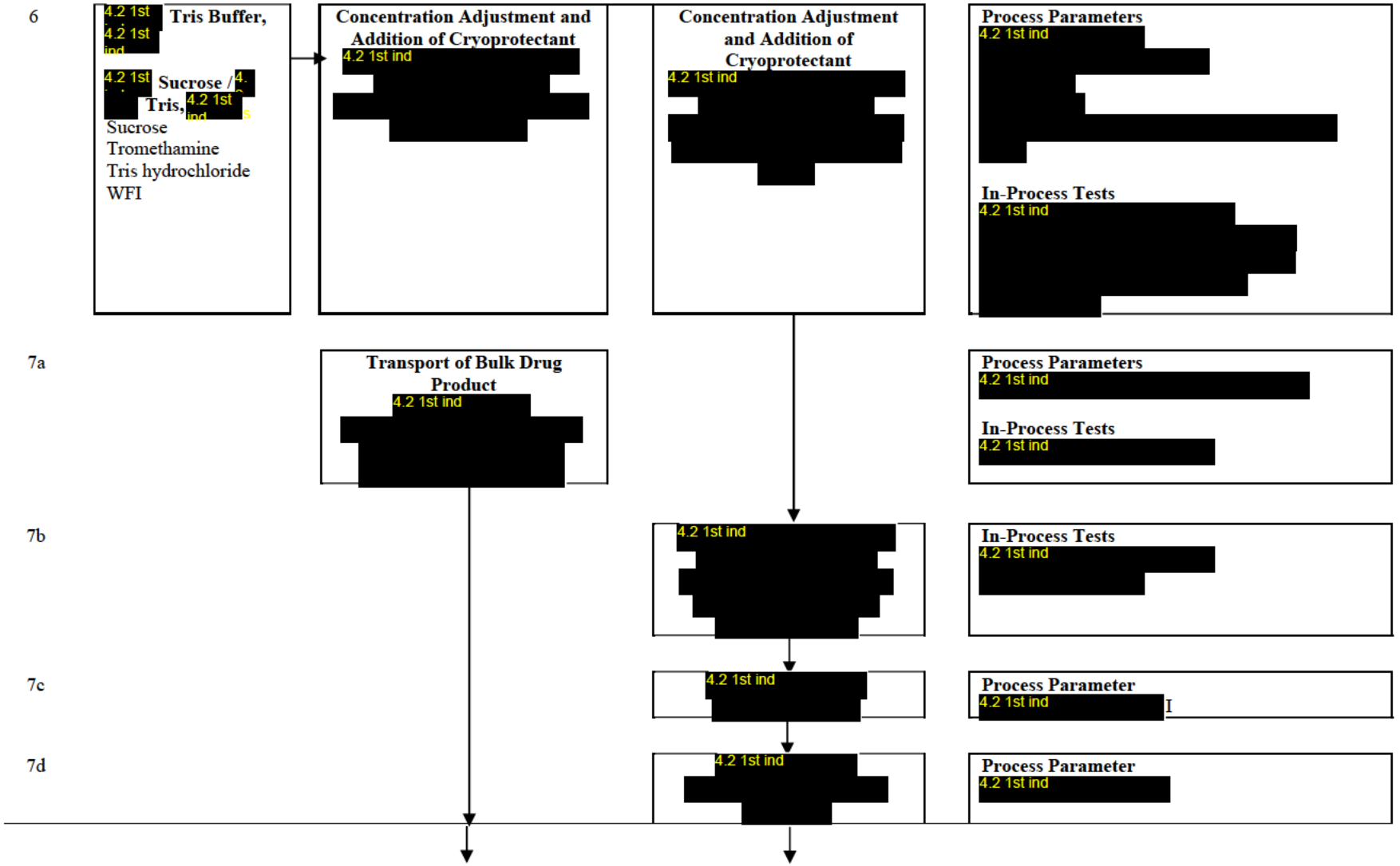

Figure 3.2.P.3.3-1. Flow Diagram of the Bivalent Drug Product Manufacturing Process and Process Controls

Abbreviations: IPT-C = In-process test for control; IPT-M = In-process test for monitoring; ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; 4.2 1st ind

3.2.P.3.3.2. LNP Production and Bulk Drug Product Formulation Process Descriptions

Drug substances are stored according to the conditions supported in the appropriate Section 3.2.S.7.1 Stability Summary and Conclusions.

3.2.P.3.3.2.1. Drug Substance Thaw

The frozen drug substances in ethylene vinyl acetate (EVA) bags may be thawed using controlled thaw equipment which consists of an automated freeze/thaw unit, agitation platform, and a validated thaw recipe. The EVA bags are placed between heat-transfer plates of the freeze/thaw unit. Once thawing is complete, the program ramps the heat transfer fluid (HTF) supply temperature down to a setpoint of 5 °C, at which time the thawed drug substance hold time begins. The controlled thaw equipment remains at a 5 °C setpoint until the EVA bags are removed. The process parameters for controlled thaw of drug substance are summarized in Table 3.2.P.3.3-1.

Table 3.2.P.3.3-1. Process Parameters for Controlled Drug Substance Thaw

Process Parameter	Acceptable Range
HTF temperature	[redacted] 4.2 1st ind
Cycle duration [redacted] 4.2 1st ind	[redacted] 4.2 1st ind

a. Target set-point
Abbreviations: HTF = heat transfer fluid

The frozen drug substances in EVA bags may also be thawed at controlled room temperature between 15-25 °C. The bags are placed in a controlled temperature room and allowed to thaw. The process parameters for controlled room temperature thaw of drug substance are summarized in Table 3.2.P.3.3-2.

Table 3.2.P.3.3-2. Process Parameters for Controlled Room Temperature Drug Substance Thaw

Process Parameter	Acceptable Range
Temperature	[redacted] 4.2 1st ind
Time	[redacted] 4.2 1st ind

a. After thawing, drug substance may be held at 2-8 °C or room temperature as described in Table 3.2.P.3.3-19
b. If the controlled room temperature thaw time exceeds 48 hours, any additional time is included in the drug substance post thaw hold time.

Alternatively, the drug substance bags manufactured at the Marburg site are directly used after storage at 2 - 8°C.

3.2.P.3.3.2.2. Pooling, Dilution, and Mixing of Drug Substances

Thawed drug substance is transferred from EVA bags to a manufacturing vessel. [redacted] 4.2 1st ind

4.2 1st ind

4.2 1st ind

Thawed drug substances are transferred from EVA bags to a mixing vessel. The two drug substances (Original and Omicron) can be added in any order. Based on a target drug product lot size, a defined quantity of one of the drug substances is transferred to the mixing vessel. The mass of the RNA in the drug substance that was transferred to the mixing vessel is calculated. Then the other drug substance is added to the mixing vessel 4.2 1st ind

Based on total RNA content and weight of the drug substances added to the vessel, the Water for Injection (WFI) amount required for dilution 4.2 1st ind is calculated. The drug substances are diluted with WFI, then mixed until homogenous. The process parameters for dilution of drug substances are summarized in Table 3.2.P.3.3-3.

Table 3.2.P.3.3-3. Process Parameters for Dilution of Drug Substances

Process Parameter	Controlled Setpoint
4.2 1st ind	4.2 1st ind
4.2 1st ind	
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind

Abbreviation: 4.2 1st ind

3.2.P.3.3.2.3. Preparation of Buffers, Organic Phase and Sucrose Solution

3.2.P.3.3.2.3.1. Preparation of 4.2 1st ind Citrate Buffer, 4.2 1st ind

The 4.2 1st ind citrate buffer, 4.2 1st ind is compounded or purchased ready to use according to the batch formula shown in Table 3.2.P.3.3-4.

Table 3.2.P.3.3-4. Batch Formula for [redacted] Citrate Buffer, [redacted]

Component	Grade	Unit Formula per 1 kg (g)
Citric acid monohydrate	USP, Ph. Eur., JP	[redacted]
Sodium citrate dihydrate	USP, Ph. Eur., JP	[redacted]
Water for Injection	USP, Ph. Eur.	[redacted]
Sodium hydroxide	Ph. Eur.	[redacted]

Abbreviations: [redacted]

The citrate buffer is mixed until homogeneous. The pH of the buffer is measured and adjusted [redacted], as necessary. The in-process test for control (IPT-C) for preparation of citrate buffer is summarized in Table 3.2.P.3.3-5. The citrate buffer is filtered [redacted] prior to use.

Table 3.2.P.3.3-5. IPT-C Test for [redacted] Citrate Buffer, [redacted]

In-process Test[a]	Acceptance Criteria
[redacted]	[redacted]

a. For internally manufactured buffer. Tests and acceptance criteria for external buffer see Section 3.2.P.3.4 Controls of Critical Steps and Intermediates - In-Process Monitoring and Control – LNP Production and Bulk Drug Product Formulation – Bivalent [BNT Marburg].

3.2.P.3.3.2.3.2. Preparation of [redacted] Tris Buffer, [redacted]

[redacted] Tris buffer, [redacted] are compounded or purchased ready to use according to the batch formula shown in Table 3.2.P.3.3-6.

Table 3.2.P.3.3-6. Batch Formula for [redacted] Tris Buffer, [redacted]

Component	Grade	Unit Formula (g/kg) for [redacted] Tris Buffer
Tromethamine	USP, Ph. Eur.	[redacted]
Tris Hydrochloride	In-house specification	[redacted]
Water for Injection	USP, Ph. Eur.	[redacted]

The [redacted] Tris buffer, [redacted] is mixed until homogeneous and measured for pH. The IPT--C test for preparation of the Tris buffer is summarized in Table 3.2.P.3.3-7.

Table 3.2.P.3.3-7. IPT-C Test for [redacted] Tris Buffer, [redacted]

In-process Test[a]	Acceptance Criteria
[redacted]	[redacted]

a. For internally manufactured buffer. Tests and acceptance criteria for external buffer see Section 3.2.P.3.4 Controls of Critical Steps and IntermediatesIn-Process Monitoring and Control – LNP Production and Bulk Drug Product Formulation – Bivalent [BNT Marburg].

The [redacted] Tris buffer, [redacted] is filtered with a [redacted] filter prior to use. The [redacted] Tris buffer, [redacted] is used for Concentration Adjustment and Addition of Cryoprotectant (Section 3.2.P.3.3.2.6) of the bulk drug product.

3.2.P.3.3.2.3.3. Preparation of the Lipid Organic Phase

The lipid organic phase is compounded according to the batch formula shown in Table 3.2.P.3.3-8.

Table 3.2.P.3.3-8. Batch Formula for Lipid Organic Phase

Component	Grade	Unit Formula per 1 kg (g)
Ethanol	USP, Ph. Eur.	[redacted]
ALC-0315	In-house specification	[redacted]
ALC-0159	In-house specification	[redacted]
DSPC	In-house specification	[redacted]
Cholesterol	USP, Ph. Eur., JP	[redacted]

Abbreviations: ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine

The lipids are thawed [redacted], if applicable. [redacted]

The process parameters for preparation of the organic phase are summarized in Table 3.2.P.3.3-9.

Table 3.2.P.3.3-9. Process Parameters for Preparation of Lipid Organic Phase

Process Parameter	Acceptable Range
[redacted]	[redacted]

a. [redacted].
b. [redacted].

Abbreviations: ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine

3.2.P.3.3.2.3.4. Preparation of 4.2 1st ind Sucrose, 4.2 1st ind Tris Solution

The 4.2 1st ind sucrose, 4.2 1st ind Tris (Sucrose/Tris) solution is compounded or purchased ready to use according to the batch formula shown in Table 3.2.P.3.3-10.

Table 3.2.P.3.3-10. Batch Formula for 4.2 1st ind Sucrose, 4.2 1st ind Tris Solution

Component	Grade	Unit Formula per 1 kg (g)
Sucrose	USP, Ph. Eur., JP	4.2 1st ind
Tromethamine	USP, Ph. Eur.	4.2 1st ind
Tris Hydrochloride	In-house specification	4.2 1st ind
Water for Injection	USP, Ph. Eur.	4.2 1st ind

The Sucrose/Tris solution is mixed until homogeneous and measured for pH. The Sucrose/Tris solution is filtered through a 4.2 1st ind filter prior to use. The IPT-C test for preparation of the Sucrose/Tris solution is summarized in Table 3.2.P.3.3-11.

Table 3.2.P.3.3-11. IPT-C Tests for 1.2 M Sucrose, 10 mM Tris Solution

In-process Test	Acceptance Criteria
4.2 1st	4.2 1st ind

a. For internally manufactured solution. Tests and acceptance criteria for external solution see Section 3.2.P.3.4 Controls of Critical Steps and Intermediates - In-Process Monitoring and Control – LNP Production and Bulk Drug Product Formulation – Bivalent [BNT Marburg].

3.2.P.3.3.2.4. Lipid Nanoparticle (LNP) Formation and Stabilization

4.2 1st ind

The process parameters for formation and stabilization of lipid nanoparticles is summarized in Table 3.2.P.3.3-12.

Table 3.2.P.3.3-12. Process Parameters for Formation and Stabilization of LNPs

Process Parameter	Acceptable Range
4.2 1st ind [redacted]	[redacted]

a. 4.2 1st ind [redacted].
Abbreviation: LNP = lipid nanoparticle

3.2.P.3.3.2.5. Buffer Exchange, Concentration and Filtration

3.2.P.3.3.2.5.1. Buffer Exchange and Concentration

For the 4.2 1st ind batch size, 4.2 1st ind [redacted] filter with a surface area of 4.2 1st ind [redacted] for the 4.2 1st ind batch size, 4.2 1st ind [redacted] with a total surface area 4.2 1st ind [redacted] are used 4.2 1st ind [redacted].

To prepare for the Buffer Exchange and Concentration operation, the 4.2 1st ind membranes are flushed 4.2 1st ind [redacted] for equilibration. When membranes are re-used between lots, the membrane and fluid path are sanitized 4.2 1st ind [redacted] prior to equilibration 4.2 1st ind [redacted].

4.2 1st ind [redacted]

Membranes that will be reused are cleaned 4.2 1st ind [redacted].

The filter information for buffer exchange and concentration is summarized in Table 3.2.P.3.3-13.

Table 3.2.P.3.3-13. Filter Properties for Buffer Exchange and Concentration

4.2 1st ind [redacted]		4.2 1st ind [redacted]	

Abbreviations: 4.2 1st ind [redacted]

The process parameters for 4.2 1st ind [redacted] filtration are summarized in Table 3.2.P.3.3-14.

Table 3.2.P.3.3-14. Process Parameters for Buffer Exchange and Concentration

Process Parameter	Acceptable Range
4.2 1st ind	4.2 1st ind
	4.2 1st ind
4.2 1st ind	4.2 1st ind
	4.2 1st ind
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind

a. 4.2 1st ind .
b. 4.2 1st ind .
c. 4.2 1st ind
d. 4.2 1st ind .

3.2.P.3.3.2.5.2. Filtration

Samples are taken for 4.2 1st ind in-process testing prior to 4.2 1st ind filtration.

The formulated drug product suspension is filtered through 4.2 1st ind filter into a transport vessel 4.2 1st ind

3.2.P.3.3.2.6. Concentration Adjustment and Addition of Cryoprotectant

Concentration adjustment can be carried out 4.2 1st ind :

- 4.2 1st ind .
- 4.2 1st ind .

3.2.P.3.3.2.6.1. Bulk Drug Product 4.2 1st ind)

Samples are taken 4.2 1st ind prior to formulation of the bulk drug product. This in-process measurement 4.2 1st ind is used to calculate the final batch weight and thereby the amount of 4.2 1st ind sucrose, 4.2 1st ind Tris solution and 4.2 1st buffer required to achieve the target drug product RNA content of 4.2 1st ind

4.2 1st ind The solution is mixed until homogeneous.

The process parameters for concentration adjustment and addition of cryoprotectant are summarized in Table 3.2.P.3.3-15.

Table 3.2.P.3.3-15. Process Parameters for Concentration Adjustment and Addition of Cryoprotectant

Process Parameter	Acceptable Range
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind

3.2.P.3.3.2.6.2. Drug Product Intermediate 4.2 1st ind

The 4.2 1st ind results in a DPI solution with an RNA concentration 4.2 1st ind.

4.2 1st ind

4.2 1st ind .

4.2 1st ind . The solution is mixed until homogeneous.

The process parameters for concentration adjustment and addition of cryoprotectant are summarized in Table 3.2.P.3.3-16.

Table 3.2.P.3.3-16. Process Parameters for Concentration Adjustment 4.2 1st ind and Addition of Cryoprotectant

Process Parameter	Acceptable Range
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind
4.2 1st ind	4.2 1st ind

3.2.P.3.3.2.7. Transport

3.2.P.3.3.2.7.1. Bulk Drug Product [redacted])

Prior to transport, the bulk drug product is filtered through [redacted] filter and filled into flexible containers (FCs) having a volume [redacted].

The FCs are stored at 2 - 8 °C until transport (shipping) to the Fill and Finish site and shipped at this temperature to the fill and finish site. The process parameters for transport of bulk drug product are summarized in Table 3.2.P.3.3-17.

Table 3.2.P.3.3-17. Process Parameters for Transport of Bulk Drug Product

Process Parameter	Acceptable Range
Storage and shipment temperature liquid bulk[a]	2 - 8 °C

a. For bulk drug product to be immediately processed at the fill and finish site.

3.2.P.3.3.2.7.2. Drug Product Intermediate [redacted]

The bulk solution with RNA concentration [redacted] is filtered through a [redacted] filter and filled into FFT bags having a volume of [redacted]. A sample for determination of the [redacted] is taken before dispensing.

The DPI is stored and shipped at -80 to -60 °C at BioNTech Marburg (Section 3.2.P.3.4 Controls of Critical Steps and Intermediates – Drug Product Intermediate [redacted] Stability Summary and Conclusion – Tris/Sucrose). The process parameters for the DPI are outlined in Table 3.2.P.3.3-18.

Table 3.2.P.3.3-18. Process Parameters for Storage and Transport of DPI (0.5 mg/mL)

Process Parameter	Acceptable Range
Storage and shipment temperature	-80 to -60 °C

3.2.P.3.3.2.8. Hold Times

The hold times of the in-process materials during LNP formation and bulk drug product formulation are provided in Table 3.2.P.3.3-19.

Table 3.2.P.3.3-19. LNP Production and Bulk Drug Product Formulation Process Hold Times

Material or In-Process Hold Description[a]	Process Steps	Target Hold Time
Drug substance thaw	Controlled thaw equipment: Time drug substance in ethylene vinyl acetate (EVA) containers is thawed [redacted]	[redacted]
	Controlled room temperature thaw: Time drug substance in EVA containers is thawed at controlled room temperature.	[redacted]
Drug substance post thaw	Maximum time that thawed BNT162b2 drug substance can be held at [redacted] C in the ethylene vinyl acetate (EVA) container including addition of DS to the vessel up to the point of dilution.	[redacted]
Drug substance post dilution [redacted]	Time from addition of WFI to drug substance until end of LNP formation step	[redacted]
Organic phase post mix [redacted]	Time from end of organic phase mixing until end of LNP formation.	[redacted]
LNP formation	Time from start of mixing aqueous and organic phases [redacted] until start of [redacted] step including collection and hold at [redacted]	[redacted]
Time of [redacted] filtration unit operation [redacted]	Time from start of [redacted] operation to end of [redacted] filtration while product is at [redacted]	[redacted]
Time post first 0.2 µm filtration at 2-8 °C	Time post [redacted] filtration until end of second [redacted] filtration with [redacted] until the start of concentration adjustment and cryoprotectant addition.	[redacted]

a. Temperature values of 15-25 °C represent room temperature
b. [redacted]
c. [redacted].
Abbreviations: RT = Room temperature; WFI = Water for Injection; LNP = lipid nanoparticle; [redacted].