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### 3.2.S.4.2. CAPILLARY GEL ELECTROPHORESIS

#### 3.2.S.4.2.1. Principle and Scope

The purpose of this analytical procedure is to determine the percent integrity of RNA in BNT162b2 drug substance (DS) and BNT162b2 drug product (DP).

This method uses capillary gel electrophoresis (CGE) to separate components based on the differential migration of RNA of different molecular weights in an applied electric field. In this procedure, the BNT162b2 test sample (TS) is subjected to a denaturant containing formamide that unfolds the RNA and dissociates non-covalent complexes. When subjected to an electric field, the denatured RNA species migrate through the gel matrix, as a function of length and size, toward the anode. An intercalating dye binds to RNA and associated fragments during migration allowing for fluorescence detection. The intact RNA is separated from any fragmented species allowing for the quantitation of RNA integrity by determining the relative percent time corrected area for the intact (main) peak.

#### 3.2.S.4.2.2. Apparatus and Equipment

The apparatus and equipment are provided in Table 3.2.S.4.2-1.

**Table 3.2.S.4.2-1. Apparatus and Equipment<sup>a</sup>**

Fragment analyzer automated capillary electrophoresis system
48-capillary array, short 33 cm
Thermomixer
Thermal cycler
Thermoshaker
96-well PCR plates
96 Deep well plates
Adhesive PCR plate seal

a. Equivalent apparatus and equipment may be used.

Abbreviations: PCR = polymerase chain reaction

#### 3.2.S.4.2.3. Reagents, Standards and Prepared Solutions

The reagents and standards are provided in [Table 3.2.S.4.2-2](#) and are of sufficient quality as to be suitable for this analytical procedure.

**Table 3.2.S.4.2-2. Reagents and Standards<sup>a</sup>**

Purified water
Nuclease-free water
Standard Sensitivity (SS) RNA kit including: RNA separating gel Intercalating dye 5X inlet buffer RNA diluent marker RNA ladder 0.25x TE rinse buffer Blank solution
5X Capillary conditioning solution
Capillary storage solution
Triton X-100
Ethanol
BNT162b2 DS reference material or other suitable DS, used as assay control
BNT162b2 DP reference material or other suitable DP, used as assay control

a. Equivalent reagents may be used.

Abbreviations: DS = drug substance; DP = drug product

The prepared solutions are provided in Table 3.2.S.4.2-3.

**Table 3.2.S.4.2-3. Prepared Solutions**

20% Triton X-100, 30% Ethanol (w/w) in nuclease-free water
RNA separation gel solution: add 1 µL intercalating dye per 10 mL RNA separating gel
Inlet buffer: add 10 mL 5X inlet buffer per 40 mL purified water

#### 3.2.S.4.2.4. Sample Preparation

DS and DP test samples (TS) are diluted with nuclease-free water to fall within instrumental limits (relative fluorescence units).

##### 3.2.S.4.2.4.1. Release of RNA from DP TS

Release of RNA from the DP lipid nano particle is required prior to analysis. Release of RNA can be performed using 4.2 1st ind.

#### 3.2.S.4.2.5. Control, Standard and Diluent Preparation

##### 3.2.S.4.2.5.1. Drug Substance Control Preparation

A DS assay control is prepared in the same manner as DS TS.

##### 3.2.S.4.2.5.2. Drug Product Control Preparation

A DP assay control is prepared in the same manner as DP TS.

### 3.2.S.4.2.5.3. Standard Preparation

An RNA ladder providing peak sizes of 15, 200, 500, 1000, 1500, 2000, 3000, 4000, and 6000 nucleotides (nt) serves as a standard for the assessment of system suitability.

### 3.2.S.4.2.5.4. Diluent Preparation

An RNA diluent marker containing a low marker (LM) peak of 15 nt and formamide is used to denature samples. No further preparation is required.

### 3.2.S.4.2.6. Procedure

#### 3.2.S.4.2.6.1. Assay Plate Preparation

The prepared DS TS and DP TS, corresponding assay control, and RNA ladder are prepared for analysis by denaturation with an RNA diluent marker. The RNA diluent marker is added to the appropriate wells, followed by the addition of the TS, RM and RNA ladder. The plate is sealed and vortexed for 2 minutes followed by centrifugation for 1 minute. The plate is then placed in a thermocycler incubator set at 70 °C with the lid set to 99 °C for 2 minutes. Once incubation is complete the plate is cooled on ice for 5 minutes followed by centrifugation for 1 minute at 200 g. Following the centrifugation each TS and assay control is mixed and separated into equal volumes in three separate wells. Any unused wells on the plate receive blank solution.

#### 3.2.S.4.2.6.2. CGE Procedure

A single injection of RNA ladder, and triplicate injections (one from each well) of TS and assay control are injected electrokinetically. Sample injection and separation are accomplished using the voltage options as described in Table 3.2.S.4.2-4.

**Table 3.2.S.4.2-4. Typical Electrophoretic Conditions and System Operating Parameters**

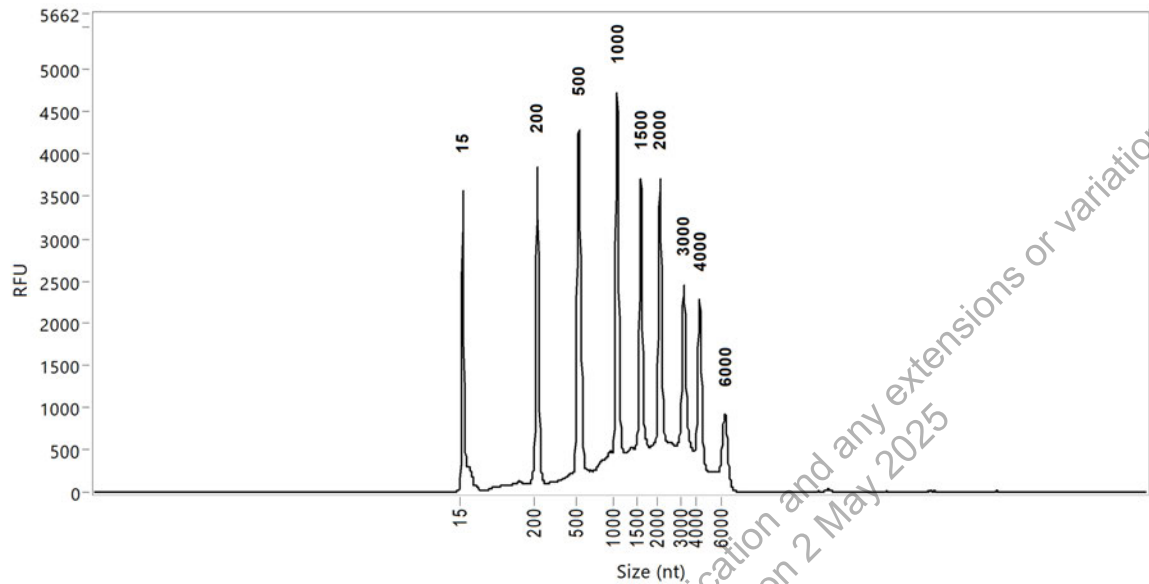
Capillary	4.2 1st ind.
Detector	
Detector light source	
Sample injection voltage	
Sample injection duration	
Separation voltage	
Separation duration	

Abbreviations: RFU = relative fluorescence units

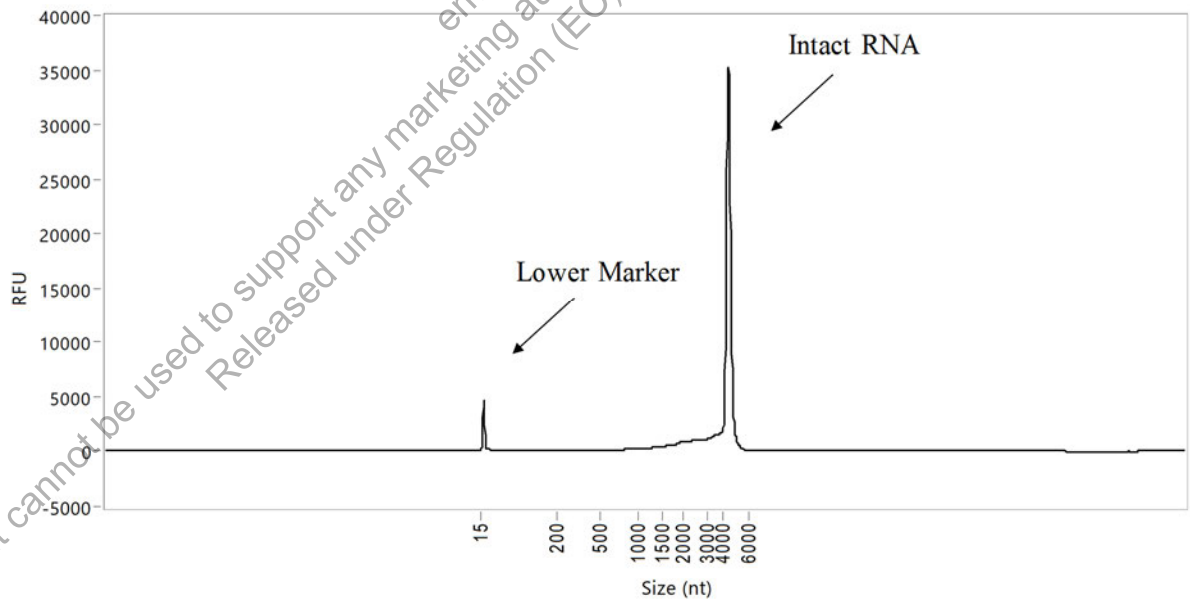
4.2 1st ind.

Representative electropherograms are shown in Figure 3.2.S.4.2-2 through Figure 3.2.S.4.2-5.

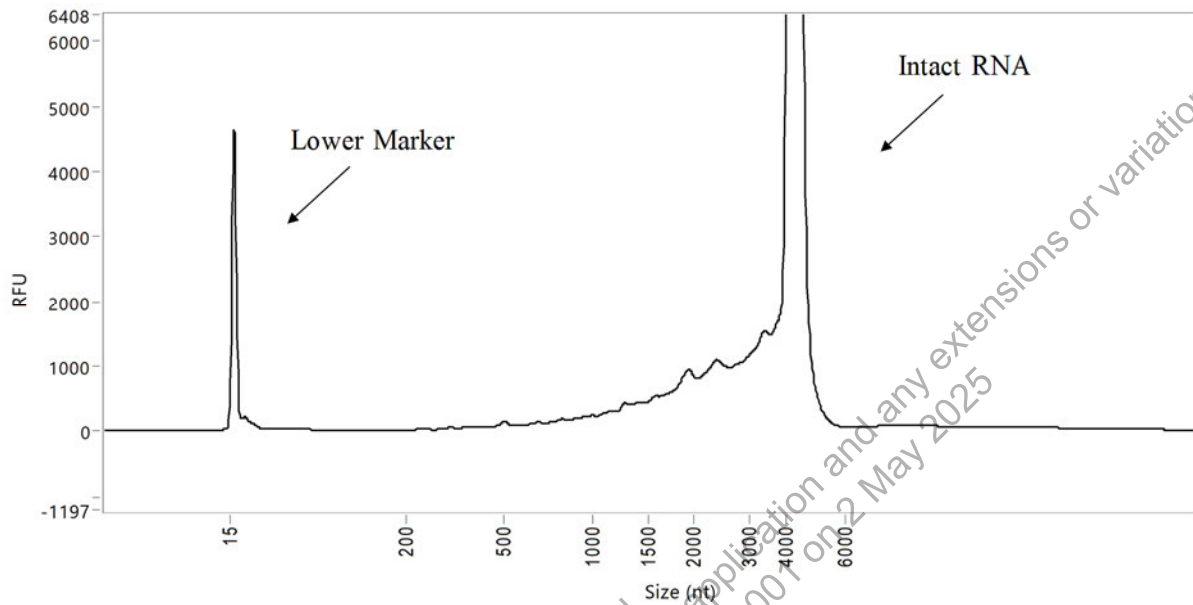
**Figure 3.2.S.4.2-1. Representative Electropherogram of RNA Ladder**



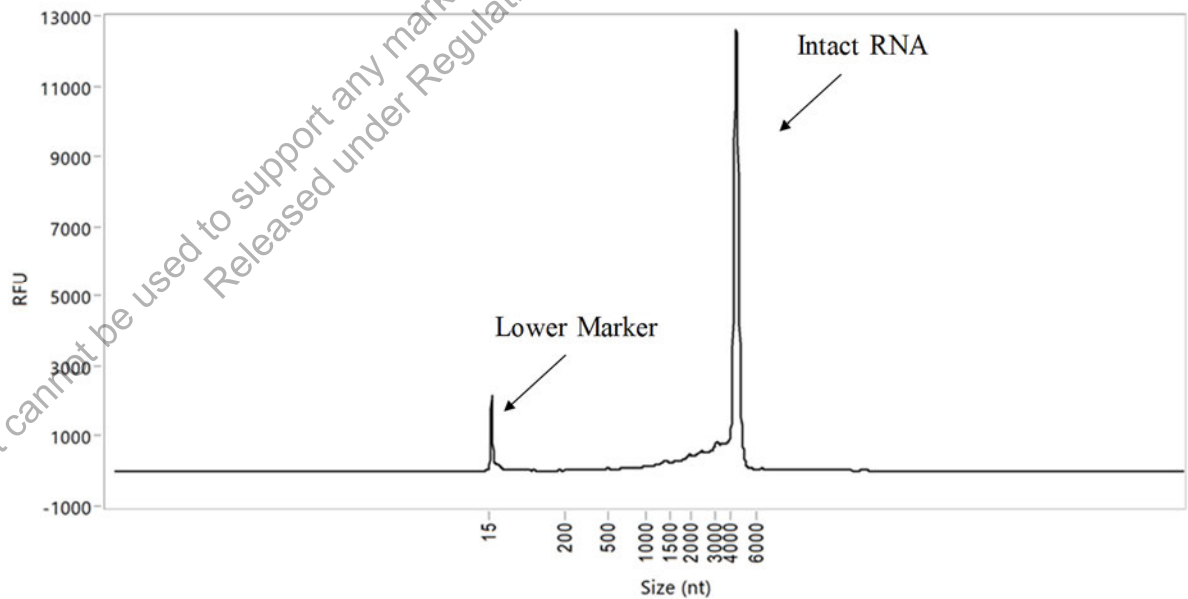
**Figure 3.2.S.4.2-2. Representative Electropherogram of BNT162b2 Drug Substance (Full View)**



**Figure 3.2.S.4.2-3. Representative Electropherogram of BNT162b2 Drug Substance (Enhanced View)**

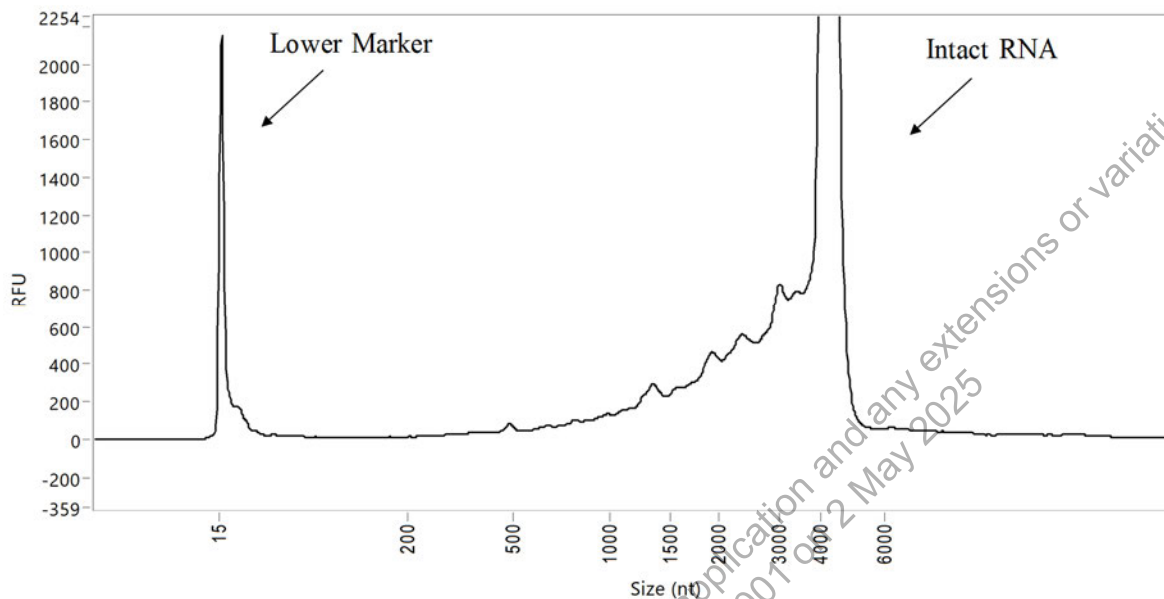


**Figure 3.2.S.4.2-4. Representative Electropherogram of BNT162b2 Drug Product (Full View)**





**Figure 3.2.S.4.2-5. Representative Electropherogram of BNT162b2 Drug Product (Enhanced View)**



#### 3.2.S.4.2.7. System Suitability, Assay and Sample Acceptance

System suitability is assessed by analysis of the RNA ladder. The criteria listed in [Table 3.2.S.4.2-5](#) must be met to demonstrate system suitability.

Assay Acceptance is assessed by analysis of the assay control. The criteria listed in [Table 3.2.S.4.2-5](#) must be met to demonstrate assay acceptance.



**Table 3.2.S.4.2-5. System Suitability, Assay and Sample Acceptance**

Material	Parameters Assessed	Acceptance Criteria
<i>System suitability</i>		
RNA ladder	<b>4.2 1st ind.</b>	
<i>Assay acceptance</i>		
Assay Control		
<i>Sample acceptance</i>		
TS		

Abbreviations: RFU = relative fluorescence units; TS = test sample; LM = low marker

- Two of the 3 replicates must meet this criterion
- Range may be updated as needed for specific instruments.

#### 3.2.S.4.2.8. Calculations

The time corrected area for the intact RNA (main) peak is calculated by dividing the peak area by the migration time. The percent RNA integrity value for each TS and assay control replicate is determined by dividing the time corrected area of the main peak by the total time corrected area for all integrated peaks and multiplying by 100.

The replicate percent RNA integrity values obtained for each TS and control are averaged to report the percent RNA integrity.

#### 3.2.S.4.2.9. Data Reporting

Provided the system suitability and assay acceptance criteria are met, the percent RNA integrity of the TS is reported.