

2.3.S.1. GENERAL INFORMATION, OMICRON (JN.1) VARIANT

2.3.S.1.1. Nomenclature

Information on the nomenclature of BNT162b2 Omicron (KP.2) drug substance is provided in Table 2.3.S.1-1.

Table 2.3.S.1-1. Nomenclature of BNT162b2 Omicron (KP.2) mRNA Drug Substance

Product code:	BNT162b2 Omicron (KP.2) mRNA Drug Substance
Laboratory code:	CCI
Chemical class:	Ribonucleic Acid (RNA)
Encoded antigen:	Viral spike protein (S1S2 protein) of the SARS-CoV-2 KP.2 sublineage (S1S2 full length protein sequence with following point mutations/deletions compared to Genebank ID QHD43416.1: CCI
CAS Registry Number:	3036385-75-5
CA Index Name:	CCI
INN	Cemivameran

2.3.S.1.2. Structure

The active component in the BNT162b2 Omicron KP.2 drug substance (DS) is a single--stranded, 5'-capped mRNA that is translated into the respective protein (the encoded antigen). Figure 2.3.S.1-1 illustrates the general structure of the antigen-encoding RNA, which is determined by the respective nucleotide sequence of the DNA used as template for *in vitro* RNA transcription. In addition to the codon-optimized sequence encoding the antigen, the RNA contains common structural elements optimized for mediating high RNA stability and translational efficiency (5'-cap, 5'-UTR, 3'-UTR, poly(A) tail; see below). Furthermore, an intrinsic signal peptide (sec) is part of the antigen-encoding regions and is translated as N-terminal tag.

Figure 2.3.S.1-1. General structure of the Omicron KP.2 mRNA

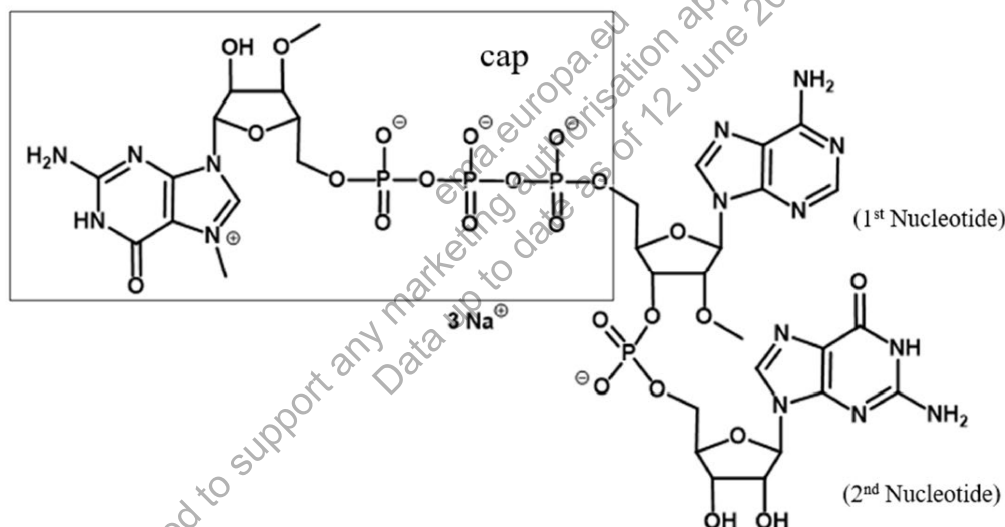


Schematic illustration of the general structure of the BNT162b2 Omicron KP.2 mRNA drug substance with 5'-cap, 5'- and 3'-Untranslated regions (5'-UTR and 3'-UTR, respectively), coding sequence for the Gene-of-Interest (GOI), and a poly(A)-tail. GOI is SARS-CoV-2 full-length spike. Individual elements are not drawn to scale compared to their respective sequence lengths.

mRNA cap

A cap1 structure $m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$ is utilized as specific capping structure at the 5'-end of the RNA drug substance (Figure 2.3.S.1-2).

Figure 2.3.S.1-2. 5'-cap analog ($m_2^{7,3'-O}Gppp(m_1^{2'-O})ApG$) for production of RNA containing a cap1 structure



The cap1 structure (i.e., containing a 2'-O-methyl group on the penultimate nucleoside of the 5'-end of the RNA chain) is incorporated into the RNA drug substance by using a respective cap analog during *in vitro* transcription. For RNAs with modified uridine nucleotides, the cap1 structure is superior to other cap structures, since cap1 is not recognized by cellular factors such as IFIT1^a and, thus, cap1-dependent translation is not inhibited by competition

^a Habjan M, Hubel P, Lacerda L, et al. Sequestration by IFIT1 Impairs Translation of 2'-O-unmethylated Capped RNA. 2013. PLOS Pathog;9(10):e1003663

with eukaryotic translation initiation factor 4E^a. In the context of IFIT1 expression, mRNAs with a cap1 structure give higher protein expression.

In addition, use of the cap1 structure leads to low amounts of uncapped transcripts^b. In general, the T7 Polymerase prefers a guanosine as priming nucleoside with the highest transcription efficiencies as compared to other starting nucleosides^c. Capping structures with a guanosine moiety compete with GTP for incorporation in the mRNA resulting in uncapped transcripts. The m₂^{7,3'-O}Gppp(m₁^{2'-O})ApG cap analog rescues transcription efficiency from templates starting with adenosines, because the ApG moiety of cap1 allows transcription initiation at the second position, a guanosine, thereby giving mainly capped mRNAs.

Further information is provided in [Section 3.2.S.1.2 Structure \[Omicron \(KP.2\) Variant\]](#).

2.3.S.1.3. General Properties

The general properties of BNT162b2 Omicron (KP.2) mRNA drug substance formulated at a target concentration of 2.25 mg/mL in DS formulation buffer (CCI) are summarized in Table 2.3.S.1-2.

Table 2.3.S.1-2. BNT162b2 Omicron (KP.2) mRNA Drug Substance General Properties

Appearance	Clear to slightly opalescent, colorless to slightly brown liquid
Specific Absorption Coefficient (260 nm)	CCI
Theoretical length^a	CCI
Theoretical mass	CCI
pH	CCI
Mechanism of Action	The nucleoside-modified messenger RNA in BNT162b2 Omicron KP.2 is formulated in lipid nanoparticles, which enable delivery of the RNA into host cells to allow expression of the SARS-CoV-2 S antigen and develop immune response. The vaccine elicits both neutralizing antibody and cellular immune responses to the spike (S) antigen, which may contribute to protection against infectious disease caused by SARS-CoV-2 virus.

^a Diamond MS. IFIT1: A dual sensor and effector molecule that detects non-2'-O methylated viral RNA and inhibits its translation. 2014. Cytokine Growth Factor Rev;25(5):543-50.

^b Trilink Patent auf CC413 cap. Accessed at <https://patentimages.storage.googleapis.com/4c/83/15/99418d175a3be2/WO2017053297A1.pdf>

^c Kuzmine I, Gottlieb PA, Martin CT. Binding of the priming nucleotide in the initiation of transcription by T7 RNA polymerase. 2003. J Biol Chem;278(5):2819-2