QUESTION 1

Information should be provided regarding the settings of the cell-free in vitro translation study. The method used should be appropriately described, including conditions for in vitro reaction, antibodies used, the nature of the positive and negative controls and choice of investigated material. Release data for the batch used in the study should be provided and additional BNT162b2 batches with different levels of RNA integrity at release, ideally having historically low and high levels of RNA integrity, should be included in the characterization exercise. (From SO1a)

RESPONSE 1

The cell-free in vitro translation is performed using the commercially available Rabbit Reticulocyte Lysate (RRL) system, which is provided by Promega. The key components for the in vitro translation reaction are the RRL, the amino acid mixtures as well as a precharged, biotin-labeled, lysine tRNA. During the translation reaction the labeled lysines are incorporated into the nascent protein chain, which enables the visualization of the resulting protein. For that, after completion of the translation reaction, the samples are subjected to a denaturing gel electrophoresis procedure (SDS-PAGE; Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) followed by the transfer to a nitrocellulose membrane via electroblotting. The visualization of the protein is achieved by binding of the biotin-labeled lysines to Streptavidin-Alkaline Phosphatase (Strep-AP) followed by colorimetric detection.

As positive control a luciferase-encoding RNA is used, which is provided within the translation kit and results in a protein band at a size of approximately 60 kDa. As negative control a sample was generated, which was subjected the complete assay workflow without any RNA in the reaction mixture to determine background signals that are not related to the RNA of the test sample.

The RNA that was used in the in vitro translation study was produced as engineering run in the BioNTech Mainz / Rentschler manufacturing node generating representative material according to the commercial process (batch no: 1071509; 37.6 L IVT reaction; 180 L drug substance; expected protein size: approx. 140 kDa). The batch data are presented in Table 1.

Table 1. Batch data of the engineering run 1071509 (BioNTech Mainz/ Rentschler)

Parameter	Result
RNA integrity	[redacted]
dsRNA	[redacted] pg dsRNA / µg RNA
Residual DNA template	[redacted] ng DNA / mg RNA
Identity of encoded RNA sequence	Identity confirmed
Appearance (Coloration)	Not more intensely colored than level 7 of brown (B) color standard
Appearance (Clarity)	[redacted] NTU
Content (RNA concentration)	[redacted] mg/mL
Bioburden	0 CFU / 10 mL
Endotoxins	< 1.0 EU/mL
pH	[redacted]
5′-Cap	[redacted]
Poly(A) tail	[redacted]

To generate different integrity levels, the RNA sample (batch 1071509) was subjected to a thermal degradation procedure, which was stopped for each level after a certain amount of incubation time. After analysis of the RNA integrity, the sample was subjected to the in vitro translation procedure as described above.

The starting material (batch 1071509) is representative of the DS, and the resulting range of RNA integrity after thermal degradation is wider than the range observed in DS batches. Therefore, the thermally degraded material was used in this characterization study.

Of note, no visual difference in the Western blot pattern can be seen in the intensity of the first two quality stages (0 min degradation ([redacted] integrity) and 2 min degradation [redacted] integrity)) indicating that no differences in the pattern using RNA in this RNA integrity range can be expected, which would be the case for any additional historical batch.

Literature References

None

SUPPORTING DOCUMENTATION

New or Replaced Supporting Documentation

None

Previously submitted supporting documentation

None