

3.2.S.4.2. DROPLET DIGITAL POLYMERASE CHAIN REACTION FOR IDENTITY (ddPCR) - [OMICRON]

3.2.S.4.2.1. Principle and Scope

The purpose of this analytical procedure is to confirm the identity of the mRNA present in variant drug substance (DS) using a one-step reverse transcription (RT) ddPCR assay.

The DS sample is diluted to an expected concentration range in the final ddPCR reaction and combined with appropriate primer/probe sets designed for confirming the presence of the variant sequence. Identity is established using a set of primers and probe designed to detect the target mRNA. Additional sets of primers and a probe may be used to test for closely related sequences, if applicable. This would confirm absence of non-target mRNA.

4.2 1st ind.

After the ddPCR reaction setup, droplets are generated and subjected to endpoint PCR thermal cycling. Droplets are analyzed to determine the concentration of the sequence which allows for identity confirmation.

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^a

4.2 1st ind.	Droplet Generator	4.2 1st ind.
4.2 1st ind.	Droplet Reader	4.2 1st ind.
Thermal Cycler		
Mini Plate Centrifuge or Centrifuge		
PCR Plate Sealer		
Sterile and nuclease free reagent reservoirs		
ddPCR 96 well semi-skirted plate,	4.2 1st ind.)
Pierceable foil heat seal,	4.2 1st ind.)
Peelable foil heat seal		
4.2 1st ind.	Droplet Generator Cartridges,	4.2 1st ind.

a. Equivalent apparatus and equipment may be used unless otherwise noted.

Abbreviations: PCR = polymerase chain reaction; ddPCR = droplet digital polymerase chain reaction

3.2.S.4.2.3. Reagents and Standards

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^{a, b}

Nuclease-Free Water	
4.2 1st ind.	Non-ionic Surfactant (100X)
TE buffer, 1X, molecular biology grade	
4.2 1st ind.	Advanced Kit for Probes, 4.2 1st ind.
Automated Droplet Generation Oil for Probes, 4.2 1st ind.	
ddPCR Droplet Reader Oil, 4.2 1st ind.	
Carrier RNA	
Internal control: a suitable DS or DP lot	
BNT162b2 Forward Primer specific to the target sequence ^b	
BNT162b2 Reverse Primer specific to the target sequence ^b	
Probe specific for the sequence specific primer set ^b	

a. Equivalent reagents may be used unless otherwise noted.

b. Primers and probes validated for the variant DS are provided with reports in Section 3.2.S.4.3 Validation of Analytical Procedures – Overview.

Abbreviations: TE = Tris-HCl containing ethylenediaminetetraacetic acid; RT-ddPCR = Reverse transcription droplet digital polymerase chain reaction; DS = Drug substance; DP = Drug product.

3.2.S.4.2.4. Sample Dilution

4.2 1st ind.

3.2.S.4.2.5. Standard and Control Solution Preparation

Internal Control (IC)

4.2 1st ind.

Negative Template Control (NTC)

Nuclease-free water is used as the NTC.

3.2.S.4.2.6. Procedure

4.2 1st ind.

4.2 1st ind.

Table 3.2.S.4.2-3. ddPCR Reaction Thermal Cycling Conditions

Temperature (°C)	Time	Cycle	Temperature Ramp Rate (°C/seconds)
46	60 minutes	1	N/A
95	10 minutes	1	N/A
95	30 seconds	40	2
58.5	1 minute		
98	10 minutes	1	N/A
4	30 minutes	1	N/A
4	Infinity	Hold	N/A
Cover Temperature: 105 °C			
Reaction Volume: 40 µL			

Abbreviations: ddPCR = Droplet digital polymerase chain reaction; N/A = not applicable.

3.2.S.4.2.7. Assay and Sample Acceptance Criteria

Assay and sample acceptance criteria are used for the assessment of the results in the analysis of the IC, NTC, and TS. The criteria listed in Table 3.2.S.4.2-4 must be met to demonstrate assay and sample acceptance for identity confirmation.

Table 3.2.S.4.2-4. Assay and Sample Acceptance for Identity Confirmation

Material	Parameters Assessed	Acceptance Criteria		
Assay acceptance				
IC	4.2 1st ind.			
NTC ^a				
Sample acceptance				
TS				

a. If testing for a closely related sequence is applicable, the results should be evaluated using the NTC criterion.

Abbreviations: IC = Internal Control, NTC = Negative Template Control, TS = Test Sample.

3.2.S.4.2.8. Calculations

The copy number per µL values obtained from the positive and negative droplet populations are reported by the instrument software program for each well.

The copies/ μ L in each TS well **4.2 1st ind.** confirms identity of the target sequence.

3.2.S.4.2.9. Data Reporting

Provided the assay and sample acceptance criteria are met, the identity results for the TS are reported.

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