



Operations Biotherapeutics Laboratories Operations Manual	
Procedure	Identification of the mRNA in modRNA BNT162b2 (1525) using RT-PCR Assay
Written	[REDACTED]
Authorised	[REDACTED]
Date issued	03 February 2021
Revision #	1

TRIM link to SOP	D21-2063055	Date of assay	20/10/2021
TRIM link to PCR template	D21-2061428	TRIM link to assay data file	D21-3234583
Operator	[REDACTED]	Checked by	[REDACTED]

Reaction mixture preparation: Prepare 15µL of reaction mixture per well plus 10% overage
 Primers and probe are provided by Pfizer and are stored in aliquots at working concentration (x10)
 Each assay requires a total of 12 wells for PCR and extraction controls plus 3 wells per test sample.

Reporting: Record the sample LIMS numbers in the table below prior to the assay. Once the assay and analysis are complete, record the assay validity criteria parameters on the worksheet below noting whether the assay is valid. For a valid assay, record the sample Ct values and whether identity has been confirmed in the table below.
 Using the Quantstudio Design & Analysis Software, update the assay results file to include the LIMS numbers of each sample (replacing the placeholder letter designation), using the "Advanced Setup" pane of the "Plate" tab. Save the updated file in the assay specific data folder in TRIM (E21-219384).
 Following completion of the assay, convert this worksheet into a .pdf and append to it the following: .pdf copies of amplification curve plots, a .pdf experiment report as generated by the Quantstudio Design & Analysis Software. Combine these into a single .pdf and file it in the assay specific data folder (E21-219384) along with the Quantstudio data file.

Reagent information

Reagent	Manufacturer and Catalogue Number	Lot Number	Expiry	Notes
QIAamp Viral RNA Mini Kit	Qiagen, 52906	166024851	2022-03-09	
Buffer AVL	Qiagen, 52906	166024291	2022-04-21	
Buffers AW1 and AW2	Qiagen, 52906	166023682 & 166019545	N/A	
Ethanol 96-100%	Supelco	K50375083828	2023/07/31	
TE Buffer	Life Technologies, AM9849	01015399	N/A	
RT-PCR-grade water	Life Technologies, AM9935	2104121	N/A	
TaqPath 1-step RT-qPCR Master Mix, CG	Applied BioSystems, A15299	2293147	2022-01-30	
ModRNA1525 RT-PCR forward primer working stock	Pfizer, 4304972	00711497-0162-M06	-	see last page for probe and primer details
ModRNA1525 RT-PCR reverse primer working stock	Pfizer, 4304972	00711497-0162-M04	-	

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ModRNA1525 RT-PCR probe working stock	Pfizer, 4316032	00711497-0162-M02	-
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Sample Preparation and Dilution

Positive PCR Control	Positive Extraction Control	Negative Extraction Control	Test Samples
<p>Dilution 1: 10µl DS RM (10µg/mL) + 90µL RT-PCR-grade water = 1µg/mL</p> <p>Dilution 2: 10µL Dilution 1 (1ug/mL) + 190µL RT-PCR-grade water = 50ng/mL = 50 pg/µL</p> <p>5µL Dilution 2 per RT-PCR well</p>	<p>Prepare with QIAamp Viral RNA Mini Kit. Lysis step composition: 140µL undiluted DP RM + 560 QIAamp Buffer AVL, without carrier RNA. Follow kit protocol Elute in 60µL Buffer</p> <p>Dilution 1: 10µL eluate + 990µL RT-PCR-grade water</p> <p>Dilution 2: 10µL Dilution 1+ 990µL RT-PCR-grade water</p> <p>5µL Dilution 2 per RT-PCR well</p>	<p>Prepare with QIAamp Viral RNA Mini Kit. Lysis step composition: 140µL TE buffer + 560 QIAamp Buffer AVL, without carrier RNA Follow kit protocol Elute in 60µL Buffer</p> <p>Dilution 1: 10µL eluate + 990µL RT-PCR-grade water</p> <p>Dilution 2: 10µL Dilution 1+ 990µL RT-PCR-grade water</p> <p>5µL Dilution 2 per RT-PCR well</p>	<p>Prepare with QIAamp Viral RNA Mini Kit. Lysis step composition: 140µL undiluted test sample + 560 QIAamp Buffer AVL, without carrier RNA Follow kit protocol Elute in 60µL Buffer</p> <p>Dilution 1: 10µL eluate + 990µL RT-PCR-grade water</p> <p>Dilution 2: 10µL Dilution 1+ 990µL RT-PCR-grade water</p> <p>5µL Dilution 2 per RT-PCR well</p>

RT-PCR Reaction Mixture

Number of wells required	15	Volume of master mix per well	15µL	Total volume of master mix required (incl.10% overage)	240	Volume of sample per well	5µL
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Component	Volume per well	Total Volume (incl. 10% overage)
Mastermix	10 µL	160
Forward Primer	1 µL	16
Reverse Primer	1 µL	16
Probe	1 µL	16
RT-PCR grade water	2 µL	32
Total volume of reaction mixture		240

Reaction mixture notes:

Mastermix used: ~~Taqman Fast Advanced / Taqman Universal II~~
4x TaqPath 1-srep Master Mix, CG

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PCR Plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	PCR Positive Control	PCR Positive Control	PCR Positive Control							Positive Extraction Control	Positive Extraction Control	Positive Extraction Control
B												
C	Sample A	Sample A	Sample A									
D	Sample B	Sample B	Sample B									
E	Sample C	Sample C	Sample C									
F	Sample D	Sample D	Sample D									
G												
H	No Template Control	No Template Control	No Template Control							Negative Extraction Control	Negative Extraction Control	Negative Extraction Control

Sample	Sample LIMS#:	Ct Value	Identity results (<i>confirmed/not confirmed</i>) (Ct must be < 32 for all sample replicates)
Sample A	2110003744	20.483, 20.347, 22.036	confirmed
Sample B			
Sample C			
Sample D			

Record Details

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Assay Validity Data

Criterion	Required value	Observed values	Validity
Ct of each Negative Extraction Control well	> 32 or undetermined	32.988, 33.941, 35.758	Valid
Ct of each No Template Control well	> 32 or undetermined	34.786, 35.120, 34.864	Valid
Ct of each PCR Positive Control well	< 32	13.471, 13.457, 13.476	Valid
Ct of each Positive Extraction Control well	< 32	18.560, 19.305, 18.490	Valid

Sample interpretation

Sequence identity of the mRNA is considered confirmed if all test sample replicates show amplification curves with a Ct value of < 32.0000. If all test sample replicates show amplification curves with a Ct value of > 32.0000 the identity is considered not confirmed. If a mixture of results (with Ct values both greater and less than 32.0000) is found for a test sample it must be repeated.

Pipettes used: **Extraction step:** 30006, 33190, 33166
PCR step: 5646, 5653, 33087

Batch details for probes and primers:

For Primer: (Merck) Sigma, Ref# VC00021, SY21020241530-088, Lot# 3026595983-000020

Rev Primer: (Merck) Sigma, Ref# VC00021, SY21020241529-078, Lot# 3026595983-000030

100 µM stock prepared as per manufacturer's instructions, 20 µL of 100 µM added to 91 µL RT-PCR

Grade water to make 18 µM working stock, MJ 06Sep21

Probe: (Applied Biosystems) ThermoFisher, Lot# 7495294-1 C1, Ref# 4316034

100 µM stock prepared as per manufacturer's instructions; 5 µL 100 µM added to 95 µL RT-PCR Grade

Water to make 5 uM working stock, MJ 27Sep21

NOTE: Validity criteria for assay has been altered as per recommendation in D21-2294359.

Criteria used to assess validity of assay are as follows:

Ct of Negative Extraction Control (NEC) more than 8 Ct higher than Ct of Positive Extraction Control (PEC):

Lowest NEC Ct value: **32.988**; highest PEC Ct value: **19.305**. Difference = **13.683** - Valid

Ct of No Template Control (NTC) more than 8 Ct higher than Ct of PCR Positive Control:

Lowest NTC Ct value: **34.786**; highest PCR Positive Control value: **13.476**. Difference = **21.31** – Valid

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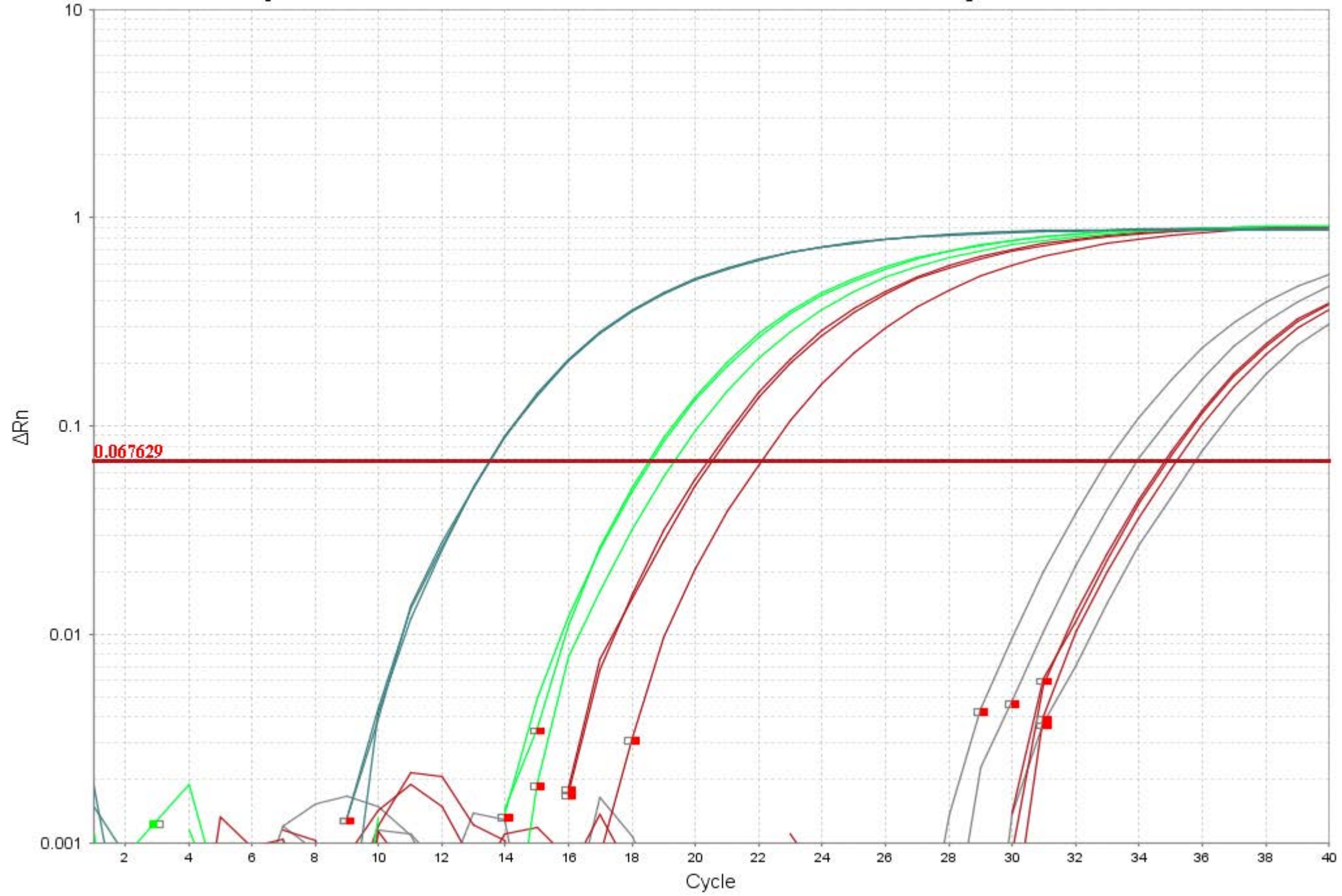
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Amplification Plot - BNT162b2 identification assay 20Oct21



■ 2110003744 ■ NEC ■ NTC ■ PEC ■ Positive Control

Experiment Results Report

Experiment Summary

Experiment Name: 2021-01-11_133749
Experiment Type: Standard Curve
Chemistry: TaqMan® Reagents
BarCode:
File Name: ID Pfizer_14.eds
Run Started: 10-20-2021 10:12:35 AEDT
Run Finished: 10-20-2021 11:18:48 AEDT
Run Duration: 66 minutes 12 seconds
Date EDS Modified: 10-20-2021 11:54:08 AEDT
Date EDS Created: 01-11-2021 15:21:26 AEDT
User Name:
Number of Wells Used: 15
Number of Wells with Results: 15
Instrument Name: QS3
Instrument Type: QuantStudio™ 3 System
Instrument Serial Number: 272322852
Model/Block Type: QuantStudio™ 3 System / 96-Well 0.2-mL Block
Quantification Cycle Setting: Ct
Stage/Step for Analysis: Stage2, Step2
Comments:



Experiment Results Report

Reagent Information

Type	Name	Part Number	Lot Number	Expiration Date
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Experiment Results Report








Results Summary

Sample	Target	Qty Mean	Qty SD	Ct Mean	Ct SD
2110003744	Target 1			20.955	0.938
NEC	Target 1				
NTC	Target 1				
PEC	Target 1			18.785	0.451
Positive Control	Target 1			13.468	0.010



Experiment Results Report

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive.. Target 1	Positive.. Target 1	Positive.. Target 1							PEC Target 1	PEC Target 1	PEC Target 1
B												
C	 211000374 Target 1	 211000374 Target 1	 211000374 Target 1									
D												
E												
F												
G												
H	 NTC Target 1	NTC Target 1	 NTC Target 1							 NEC Target 1	 NEC Target 1	NEC Target 1



Experiment Results Report

Results Table

Well	Sample	Target	Task	Ct	Ct Mean	Ct SD	Qty	Qty Mean	Qty SD	Ct Threshold	Baseline Start	Baseline End	Cq Conf
A1	Positive Control	Target 1	U	13.471	13.468	0.010				0.068	3	9	0.934
A2	Positive Control	Target 1	U	13.457	13.468	0.010				0.068	3	9	0.963
A3	Positive Control	Target 1	U	13.476	13.468	0.010				0.068	3	9	0.933
H11	NEC	Target 1	N	33.941						0.068	3	30	0.969
H10	NEC	Target 1	N	32.988						0.068	3	29	0.963
A10	PEC	Target 1	U	18.560	18.785	0.451				0.068	3	14	0.956
A11	PEC	Target 1	U	19.305	18.785	0.451				0.068	3	15	0.956
H12	NEC	Target 1	N	35.758						0.068	3	31	0.966
A12	PEC	Target 1	U	18.490	18.785	0.451				0.068	3	15	0.951
C1	2110003744	Target 1	U	20.483	20.955	0.938				0.068	3	16	0.955
C2	2110003744	Target 1	U	20.347	20.955	0.938				0.068	3	16	0.954
C3	2110003744	Target 1	U	22.036	20.955	0.938				0.068	3	18	0.953
H1	NTC	Target 1	N	34.786						0.068	3	30	0.937
H3	NTC	Target 1	N	34.864						0.068	3	31	0.945
H2	NTC	Target 1	N	35.120						0.068	3	31	0.930

Legend: S = Standard, N = Non Template Control, U = Unknown, UND. = Undetermined



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Experiment Results Report

QC Summary

Total Wells:96 Processed Wells:15 Targets Used:1 Well Setup:15 Flagged Wells:7 Samples Used:5

Flag	Description	Frequency	Locations
AMPNC	Amplification in negative control	4	H11, H10, H1, H3
BADROX	Bad passive reference signal	0	
BLFAIL	Baseline algorithm failed	0	
CQCONF	Low Cq confidence	0	
CTFAIL	Ct algorithm failed	0	
DRNMIN	Define acceptable delta Rn based on Ct range	0	
EXPFAIL	Exponential algorithm failed	0	
HIGHSD	High standard deviation in replicate group	3	C1, C2, C3
HIGHSD	High standard deviation in replicate group	0	
NOAMP	No amplification	0	
NOISE	Noise higher than others in plate	0	
NOSIGNAL	No signal in well	0	
OFFSCALE	Fluorescence is offscale	0	
OUTLIERRG	Outlier in replicate group	0	
PRFDROP	Passive reference signal changes near Ct	0	
PRFLOW	Low passive reference signal	0	
SPIKE	Noise spikes	0	
THOLDFAIL	Thresholding algorithm failed	0	

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