

<b>Owner:</b> [REDACTED]	<b>Number:</b> Bio-BPC-Form-11
<b>Author:</b> [REDACTED]	<b>Version:</b> 1
<b>Active:</b> 15/06/2021	<b>Review:</b> <QPulse_DocReviewDate>
<b>Title:</b> Appendix 1 - Fragment Analyzer Worksheet - Pfizer COMIRNATY	

## Worksheet for Fragment Analyzer - RNA Integrity

Test Details			
<b>SOP QPulse #</b>	Bio-BPC-Method-26	<b>Analysist</b>	[REDACTED]
<b>TRIM link to data files</b>	D21-3280419, D21-3280418	<b>Test Date</b>	1/11/2021

Pipettes & Equipment	
Name	LIMS#
30-300 µL 12 channel pipette	N/A
p10 pipette	32835
p50 pipette	N/A
p100 pipette	32792
p200 pipette	5649
Thermomixer	23660
Thermocycler	N/A
P20	32891

Reagents & Consumables			
Details	Catalog #	Lot/Batch Number	Expiry date
48-Capillary Array, short 33 cm	A2300-4850-3355	022621-27sfs	N/A
Standard Sensitivity (SS) RNA kit Part 1 stored at 2-8°C	DNF-471-0500	0006609959	10/03/2022
<i>Extra Blank solution</i>	<i>DNF-300-0008</i>	6594431	3/03/2022
Standard Sensitivity (SS) RNA kit Part 2 stored at -20°C (Diluent Marker & Intercalating dye)	Enter text.	Enter text.	Enter a date.
<i>Extra Diluent marker</i>	<i>DNF-369-0004</i>	0006602443	7/04/2023
Standard Sensitivity (SS) RNA kit Part 3 stored at -70°C (RNA Ladder)	DNF-382-U020	0006600148	29/03/2022
Capillary conditioning solution	DNF-475-0100	6598614	22/03/2022
DEPC water	AM9961	2004017	N/A
20% Triton-X100 / 30% Ethanol solution	In house	MC1SEP21-01	1/02/2022
Enter text.	Enter text.	Enter text.	Enter a date.
Intercalating dye	Dnf-600-u030	6603014	9/04/2022
Enter text.	Enter text.	Enter text.	Enter a date.

Reagent Preparation			
REAGENT	STORAGE	Date Prepared	Expiry
<b>Inlet Buffer</b> - 10 mL 5X inlet buffer (fridge) - 40 mL MilliQ Water	Deep well plate, RT (Drawer B)	1/11/2021	2/11/2021  Prepare fresh
<b>Rinse Buffer</b> 200 µL per well of 0.25X TE buffer (stored in fridge, allow to come to RT) Can also use DEPC water	96 well plate, RT (Drawer M)	1/11/2021	2/11/2021  Prepare fresh
<b>Capillary Storage Buffer</b> 100 µL per well capillary storage solution (RT)	96 well plate, RT (Drawer 3)	26/10/2021	9/11/2021  2 weeks
<b>Capillary Conditioning solution</b> - 6 mL 5X capillary conditioning Soln (RT) - 24 mL Milli-Q Water	250 mL tube, RT (Capillary conditioning line)	1/11/2021	2/11/2021  2 weeks
<b>RNA Separation gel</b> - 23 mL RNA separating gel (fridge) - 2.3 µL Intercalating dye (-20°C)	50 mL tube, RT (Gel line 2)	1/11/2021	3/11/2021  48 hours
<b>Empty waste tray and waste bottle</b> Reagents can be scaled up if required – this table provides the minimum for a single run. Keep all samples, RNA ladder (-70°C), and RNA diluent marker (-20°C) on ice until ready for use Allow separating gel, blank solution, rinse buffer, inlet buffer (all stored in fridge), and the intercalating dye (-20°C) to come to RT before use  Blank solution can be replaced with diluent marker. All wells need to contain a peak for capillary alignment.			

**96 well Plate Layout**

	1	2	3	4	5	6	7	8	9	10	11	12
A	BF-25	BF-25	BF-25	BF-25	BF-25	S6a	S5a	S4a	S3a	S2a	S1a	RM
B	BF-25	BF-25	BF-25	BF-25	BF-25	S6b	S5b	S4b	S3b	S2b	S1b	RM
C	BF-25	BF-25	BF-25	BF-25	BF-25	S6c	S5c	S4c	S3c	S2c	S1c	RM
D	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	BF-25	L

**S1-6** = Samples in triplicate (a, b or c),  
**RM** = Reference material  
**BF-25** = Blank solution provided in kit,  
**L** = RNA ladder

This worksheet assumes the maximum of 6 samples per test. Any samples not included in the test must be crossed off the plate layout, and results table below

System Suitability Criteria – RNA Ladder			
Plate location (wells)	D12		
Parameter	Limits	Results	Comments
RNA ladder profile	Visually comparable to figure 4 of SOP	ok	PASS
All peaks present	15 200 500 1000 1500 2000 3000 4000 6000 nt	ok	PASS
Peak heights	<60000 RFU	ok	PASS
Assay Acceptance Criteria – Reference Material			
Plate location (wells)	A12 B12 C12		
LIMS #	2108002914		
BATCH #	EE8493		
EXPIRY	5/02/2022		
Parameter	Limits	Results	Comments
Profile	Visually comparable to DP electropherogram in SOP	Ok/ok/ok	PASS
Migration time	Approximately comparable to profile in SOP	3981/4000/3981	PASS
Lower marker present	LM peak	Ok/ok/ok	PASS
Peak heights	5000-600000 for 2/3 replicates	11936/11270/10990	PASS
No negative peaks or baseline shifts	No significant peaks/shifts	Ok/ok/ok	PASS
Reference Material Dilutions / Calculation / Notes			
thaw date: 19/10/21 270ng/uL = 20 uL of 530 ng/uL master stock + 19 uL DEPC water 90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution  The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919			

Sample 1 Details	
Plate location (wells)	A11 B11 C11
LIMS #	2110003905
BATCH #	FL7649
EXPIRY	28/02/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	4000/3981/3962	PASS
Lower marker	LM must be present	Ok/ok/ok	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	14106/15399/14335	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	■	64.50	1.23	1.91	PASS
% Late Migrating Species		2.87	0.25	8.78	

Sample Dilutions / Calculation / Notes
<p>opened for the first time 01/11/21, stored at 2-8C                      270ng/uL = 20 uL of 500 ng/uL master stock + 17uL DEPC water                      90 ng/uL = 20 uL of 270 ng/uL + 40 uL 20%Tx100 solution</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
PASS	
Analysist	■
Checked by	■

Sample 2 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	■	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 3 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	PASS
Lower marker	LM must be present	Enter text.	PASS
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	PASS

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	■	81.03	1.24	1.53	PASS
% Late Migrating Species		0.87	0.21	24.02	

Sample Dilutions / Calculation / Notes
<p>Thaw date – 25/10/2021 – stored cell culture fridge , opened for the first time 25/10/21, stored at 2-8C</p> <p>60 ng/uL = 20 uL of 200 ng/uL DP + 40 uL %Tx100 solution/30% ethanol</p> <p>The following method modifications have been used: RNA denaturation step performed using a thermoblock as outlined in the following report: D21-3185919</p>

Sample Results	
<b>Choose an item.</b>	
Analysist	■
Checked by	Enter text.

Sample 4 Details	
Plate location (wells)	A8 B8 C8
LIMS #	2110003932
BATCH #	000061A
EXPIRY	5/05/2022

Sample Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	3981/3981/3942	Choose an item.
Lower marker	LM must be present	Ok/ok/ok	Choose an item.

Peak heights	5000-60000 RFU for 2/3 replicates	13683/11870/13713	Choose an item.
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Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	■	Enter text.	Enter text.	Enter text.	Choose an item.
% Late Migrating Species		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes
Enter text.

Sample Results	
Choose an item.	
Analysist	Enter text.
Checked by	Enter text.

Sample 5 Details	
Plate location (wells)	Choose an item.
LIMS #	Click or tap here to enter text.
BATCH #	Click or tap here to enter text.
EXPIRY	Enter date.

Acceptance Criteria			
Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
Parameters	Limits	Results			Comments
		Average	SD	%RSD	
% RNA Integrity	■	Enter text.	Enter text.	Enter text.	Choose an item.

<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	
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Sample Dilutions / Calculation / Notes					
Enter text.					

Sample Results					
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**Choose an item.**

<b>Analysist</b>	Enter text.				
<b>Checked by</b>	Enter text.				

Sample 6 Details					
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<b>Plate location (wells)</b>	Choose an item.				
<b>LIMS #</b>	<b>Click or tap here to enter text.</b>				
<b>BATCH #</b>	Click or tap here to enter text.				
<b>EXPIRY</b>	Enter date.				

Acceptance Criteria			
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Parameter	Limits	Results	Comments
Migration time	Comparable to RM	Enter text.	Choose an item.
Lower marker	LM must be present	Enter text.	Choose an item.
Peak heights	5000-60000 RFU for 2/3 replicates	Enter text.	Choose an item.

Test Results					
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Parameters	Limits	Results			Comments
		Average	SD	%RSD	
<b>% RNA Integrity</b>	■	<b>Enter text.</b>	Enter text.	Enter text.	Choose an item.
<b>% Late Migrating Species</b>		Enter text.	Enter text.	Enter text.	

Sample Dilutions / Calculation / Notes					
Enter text.					

Sample Results					
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**Choose an item.**



<b>Analysist</b>	
<b>Checked by</b>	Enter text.

<b>Notes</b>
Enter text.

