Australian Government
Department of Health
Therapeutic Goods Administration

Laboratories Branch

Operations	Biotherapeutics Laboratories Operations Manual
Procedure	PCR – Form – Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence Worksheet
Written	[redacted]
Authorised	[redacted]
Date issued	10 August 2021
Revision #	3

Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence - Worksheet

Operator	[redacted]	**Date of assay**	19/10/2021
Checked by	[redacted]	**TRIM link to assay data file**	el://D21-3232383?db=A7&open

Results Summary

Sample Number	LIMS#	Total RNA	% Encapsulation
1	2110003744	[redacted] mg/mL	[redacted]
2			
3			

Reagent records

Reagent	Manufacturer and Catalogue Number	Lot Number	Expiry	Notes
Drug Substance Reference Material	PF-07305885	20Y513C701-WRM	N/A	
Quant-iT RiboGreen RNA Reagent	Thermo Fisher, R11491	2181574	N/A	
Triton X-100 10% Solution in Water	Thermo Scientific 85111	VJ309153	-	
TE Buffer	Invitrogen, AM9935	01015399	-	

Additional notes

Standard Curve Preparation

Prepare the standard curve material using aliquotted DS RM at 10µg/mL, diluted according to the table below

Standard ID	In-Plate RNA Concentration (ng/mL)	Volume of DS RM at 10µg/mL (µL)	Volume of TE buffer (µL)
STD 1	2500	150	150
STD 2	2000	100	150
STD 3	1600	80	170
STD 4	1200	120	380
STD 5	800	100	525
STD 6	400	100	1150
STD 7	200	50	1200
STD 8	0	0	400

Drug product sample preparation - Pfizer Comirnaty

Tube 1: 20µL Drug Product (c. 500µg/mL) + 180µL TE buffer
Tube 2: 60µL Tube 1 (c. 50µg/mL) + 240µL TE buffer

TE Sample: 72µL Tube 2 (10µg/mL) + 378 TE buffer - Dilution factor: 312.5
Triton X-100 Sample: 18µl Tube 2 (10µg/mL) + 432µL 1% Triton buffer - Dilution factor: 1250

Redacted under section 22 of the FOI Act

Buffer Formulations

1% Triton X-100

Component	Volume Added
10% Triton X-100	3 mL
TE Buffer	27 mL

2% Triton X-100

Component	Volume Added
10% Triton X-100	6 mL
TE Buffer	24 mL

Quant-IT Ribogreen Working Solution

Component	Volume Added
Quant-IT Ribogreen Reagent	50 µL
TE Buffer	10 mL

Pipettes used for assay

32837, 32892, 32792, 30016

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	50µL **STD 8** 50µL TE		50µL **STD 8** 50µL 2% Triton									
B	50µL **STD 7** 50µL TE		50µL **STD 7** 50µL 2% Triton		100µL **Sample 1** in **TE** buffer	100µL **Sample 2** in **TE** buffer	100µL **Sample 3** in **TE** buffer					
C	50µL **STD 6** 50µL TE		50µL **STD 6** 50µL 2% Triton									
D	50µL **STD 5** 50µL TE		50µL **STD 5** 50µL 2% Triton									
E	50µL **STD 4** 50µL TE		50µL **STD 4** 50µL 2% Triton		100µL **Sample 1** in **1% Triton** buffer	100µL **Sample 2** in **1% Triton** buffer	100µL **Sample 3** in **1% Triton** buffer					
F	50µL **STD 3** 50µL TE		50µL **STD 3** 50µL 2% Triton									
G	50µL **STD 2** 50µL TE		50µL **STD 2** 50µL 2% Triton									
H	50µL **STD 1** 50µL TE		50µL **STD 1** 50µL 2% Triton									

Plus 100µL of Ribogreen working solution to each assay well

After completing the assay, convert the above to .pdf and combine it with the .pdf report generated from the SoftmaxPro assay data file to form a single file containing both the worksheet and results. File the combined .pdf and the raw softmax data (.sda) in the assay specific data folder (E21-207399)

Record Details	PCR - Form - Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence Worksheet		
Last Editor	[redacted]	Edit Date	10/08/2021 9:35
Print Date	10/08/2021 9:35 AM		AM Page 3 of 3

Plate1

	1	2	3	4	5	6	7	8	9	10	11	12
A	8.966	9.104	26.396	26.239	8.395	0.364	0.383	0.349	0.392	0.337	0.368	0.375
B	160.94	158.51	142.58	146.57	142.66	0.323	0.352	0.383	0.393	0.347	0.389	0.349
C	354.67	356.27	293.59	300.29	144.11	0.378	0.338	0.372	0.382	0.350	0.390	0.344
D	685.26	700.39	569.49	571.90	139.11	0.326	0.342	0.371	0.381	0.370	0.392	0.379
E	1069.7	1062.6	886.08	887.11	349.20	0.342	0.367	0.362	0.388	0.370	0.403	0.366
F	1345.3	1357.7	1092.6	1116.8	351.97	0.304	0.346	0.347	0.401	0.355	0.413	0.355
G	1729.0	1744.2	1464.2	1475.1	344.51	0.347	0.367	0.361	0.418	0.350	0.388	0.363
H	2086.3	2137.0	1840.0	1869.1	8.767	0.346	0.335	0.395	0.371	0.353	0.309	0.385

Settings Information
Endpoint
Fluorescence
Ex, Auto Cutoff, Em
Lm1 485, 515, 528
PMT and Optics
Auto
6 Flashes/read
Read From Top
More Settings
Shake Once
Calibrate On
Column Priority

Read Information
SpectraMax M5e
ROM v3.0.22 16Feb11
Start Read : 5:02 PM
19/10/2021

Mean Temperature : 24.3 °C

Reduction Settings
Wavelength Combination : !Lm1

Results Summary

Results Summary **LIMS# 33237** **Version 1, Feb2021** **Validated 01/02/21 - Next Due 01/02/22**
Pfizer - BNT162b2
Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence

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Assay Validity Criteria		Observed Value	Validity
Coefficient of determination (R^2) for TE standard curve	≥ 0.98	= 0.999	**VALID**
Coefficient of determination (R^2) for TX standard curve	≥ 0.98	= 0.999	**VALID**
%RSD for all standard curve points (point 8, 0ng/mL, excluded)	≤ 20%	Pass	**VALID**
Monotonous increase over TE standard curve	Present	Present	**VALID**
Monotonous increase over TX standard curve	Present	Present	**VALID**

Sample 1: **VALID**

Free RNA = [redacted] ng/mL

Total RNA = ng/mL = [redacted] **mg/mL**

Encapsulation = [redacted] %

Sample 2: **INVALID**

Free RNA = [redacted] ng/mL

Total RNA = [redacted] ng/mL = [redacted] **mg/mL**

Encapsulation = [redacted] %

Sample 3: **INVALID**

Free RNA = [redacted] ng/mL

Total RNA = [redacted] ng/mL = [redacted] **mg/mL**

Encapsulation = [redacted] %

Sample Validity Criteria:

%RSD of replicates must be < 20% in both TE and Triton X-100 buffers

Sample fluorescence values must fall within the range of their respective standard curves

DilutionFactorTE = 312.5

DilutionFactorTX100 = 1250

Spreadsheet validation procedure:

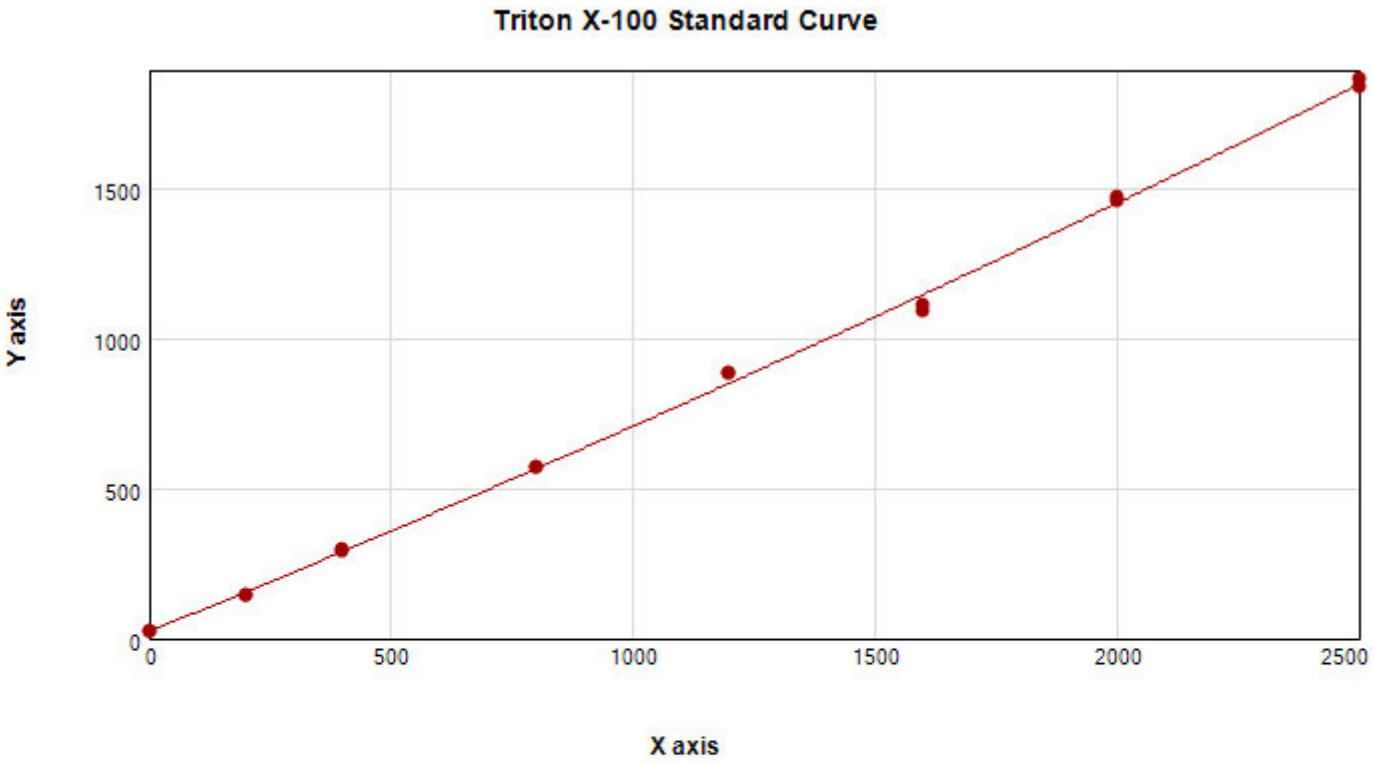
Open the active copy of Softmax Pro template "Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence.spr".

Open spreadsheet validation data set (TRIM link: D21-2134364) and copy well data from Plate 1. Paste well data into Plate 1 in the Softmax Pro Template undergonig validation.

Generate a .pdf printout. File the .pdf in the validation folder in TRIM (E21-212109), using thefile name "Spreadsheet validation results - LIMS#33237 - DDMMMYY - username.pdf"

If the calculated values and validity status are consistent between the generated report and those recorded in the validation dataset report (TRIM link: D21-2134332) the template is considered valid, and a "PASS" result is to be entered into qLIMS. Update the validation date and due date in the template and submit a zipped version of the template for storage in q-pulse.

In the event of inconsistent results, a "FAIL" result is to be enetered into qLIMS and the issue is to be reported to the PCR Unit Manager.

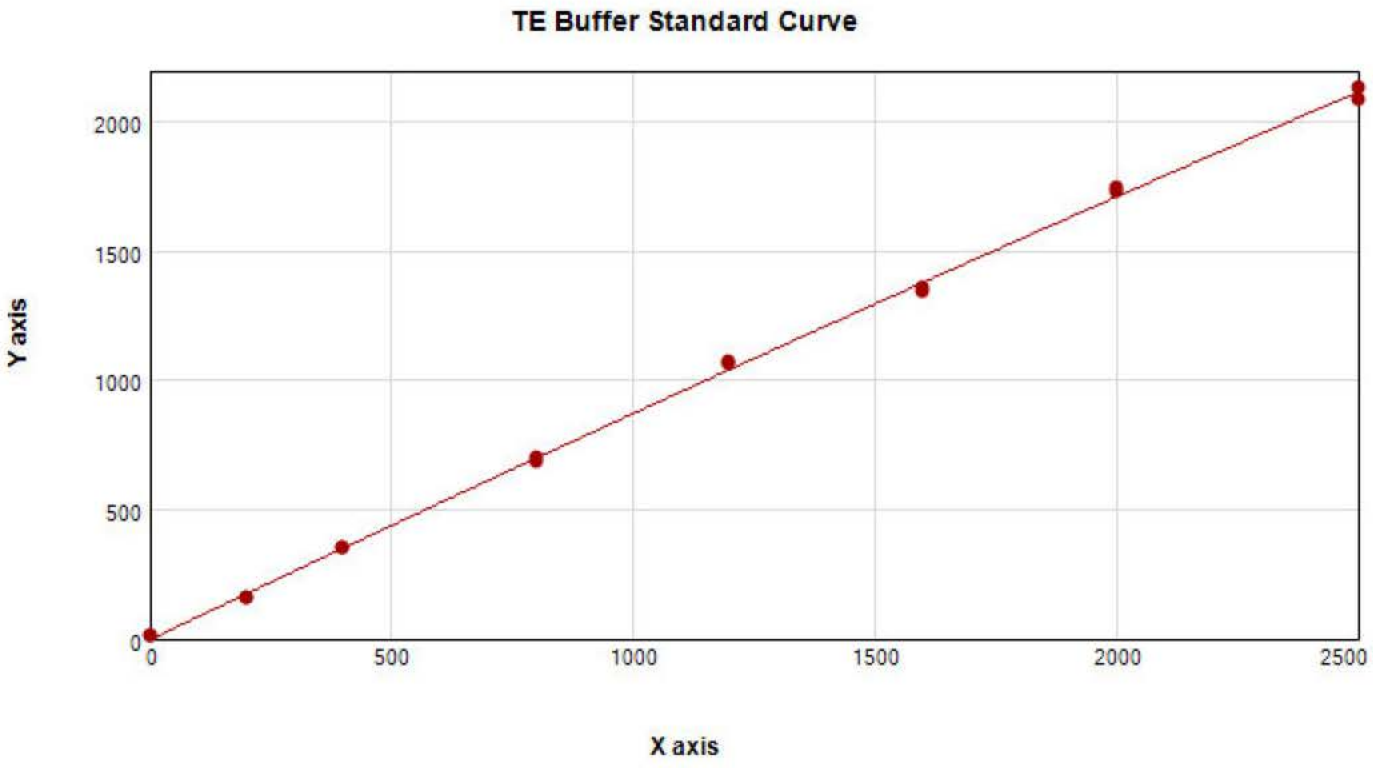


● **Plot2** (Standard Curve, Triton X-100 : Values vs Concentr...)

Curve Fit Results ▲

Curve Fit : Quadratic $y = A + Bx + Cx^2$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 R^2 = 0.999	A	24.41	12.76	[-3.155, 51.98]
	B	0.655	0.027	[0.598, 0.713]
	C	3.02e-5	1.06e-5	[7.43e-6, 5.31e-5]



Curve Fit Results ▲

Curve Fit : Quadratic $y = A + Bx + Cx^2$

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1 R^2 = 0.999	A	-2.588	11.67	[-27.80, 22.62]
	B	0.894	0.024	[0.841, 0.946]
	C	-1.81e-5	9.66e-6	[-3.90e-5, 2.75e-6]

Sample 1, Triton X-100 Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTX1 (ng/mL)	Std.Dev.	CV%
01	E5	[redacted]					5.509	1.139
	F5							
	G5							

Validity by RSD = VALID

Validity by range of standard curve = VALID

Sample 1, TE Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTE1 (ng/mL)	Std.Dev.	CV%
01	B5	[redacted]					2.896	1.785
	C5							
	D5							

Validity by RSD = VALID

Validity by standard curve range = VALID

Sample 2, Triton X-100 Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTX2 (ng/mL)	Std.Dev.	CV%
01	E6						0.036	0.098
	F6							
	G6							

Validity by RSD = VALID

Validity by range of standard curve = INVALID

Sample 2, TE Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTE2 (ng/mL)	Std.Dev.	CV%
01	B6						0.035	1.055
	C6							
	D6							

Validity by RSD = VALID

Validity by range of standard curve = INVALID

Sample 3, Triton X-100 Buffer

Sample	Well	Values	Mean Values	Result	MeanResult	ConcTX3 (ng/mL)	Std.Dev.	CV%
01	E7						0.019	0.050
	F7							
	G7							

Validty by RSD = VALID

Validity by range of standard curve = INVALID

Sample 3, TE Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTE3 (ng/mL)	Std.Dev.	CV%
01	B7						0.008	0.246
	C7							
	D7							

Validity by RSD = VALID

Validity by Standard Curve Range = INVALID

Standard Curve, Triton X-100

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngV...	200ng...	400ngVal...	800ngVal...	1200ng...	1600ng...
01	A3	0.000	26.396	26.318	0.111	0.422	1	0	26.318	0.000	0.000	0.000	0.000	0.000
	A4		26.239											
02	B3	200.000	142.582	144.578	2.823	1.952	0	0	0.000	144.578	0.000	0.000	0.000	0.000
	B4		146.574											
03	C3	400.000	293.590	296.942	4.740	1.596	0	0	0.000	0.000	296.942	0.000	0.000	0.000
	C4		300.293											
04	D3	800.000	569.488	570.697	1.709	0.299	0	0	0.000	0.000	0.000	570.697	0.000	0.000
	D4		571.905											
05	E3	1200.000	886.079	886.594	0.729	0.082	0	0	0.000	0.000	0.000	0.000	886.594	0.000
	E4		887.110											
06	F3	1600.000	1092....	1104.709	17.079	1.546	0	0	0.000	0.000	0.000	0.000	0.000	1104.709
	F4		1116....											
07	G3	2000.000	1464....	1469.648	7.770	0.529	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G4		1475....											
08	H3	2500.000	1840....	1854.556	20.541	1.108	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H4		1869....											

2000ng...	2500ng...
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
1469.6...	0.000
0.000	1854.556

Validation Check Sum by RSD = 0 MaxTXStdCve = 1854.556 MinTXStdCve = 26.318 Monotonic increase = VALID

Standard Curve, TE Buffer

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngVal...	200...	400ngV...	800ng...	1200ng...	1600n...
01	A1	0.000	8.966	9.035	0.098	1.080	1	0	9.035	0.000	0.000	0.000	0.000	0.000
	A2		9.104											
02	B1	200.000	160.936	159.722	1.717	1.075	0	0	0.000	159....	0.000	0.000	0.000	0.000
	B2		158.508											
03	C1	400.000	354.674	355.473	1.130	0.318	0	0	0.000	0.000	355.473	0.000	0.000	0.000
	C2		356.272											
04	D1	800.000	685.259	692.823	10.698	1.544	0	0	0.000	0.000	0.000	692.823	0.000	0.000
	D2		700.388											
05	E1	1200.000	1069....	1066.160	5.014	0.470	0	0	0.000	0.000	0.000	0.000	1066.160	0.000
	E2		1062....											
06	F1	1600.000	1345....	1351.519	8.751	0.648	0	0	0.000	0.000	0.000	0.000	0.000	1351....
	F2		1357....											
07	G1	2000.000	1729....	1736.593	10.687	0.615	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G2		1744....											
08	H1	2500.000	2086....	2111.664	35.854	1.698	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H2		2137....											

2000n...	2500n...
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
0.000	0.000
1736....	0.000
0.000	2111.6...

Validation Check Sum by RSD = 0 MaxTEStdCve = 2111.664 MinTEStdCve = 9.035 Monotonic Increase = VALID