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 PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence Worksheet
Written	[redacted]
Authorised	[redacted]
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Revision #	2

Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence - Worksheet

Operator	[redacted]	**Date of assay**	19/07/2021
Checked by		**TRIM link to assay data file**	el://D21-2860013?db=A7&open

Results Summary

Sample Number	LIMS#	Total RNA	% Encapsulation
1	2107002694	[redacted]	[redacted]
2			
3			

Reagent records

Reagent	Manufacturer and Catalogue Number	Lot Number	Expiry	Notes
PF-07305885 Drug Substance Reference Material	Pfizer, 20Y513C201-RM	20Y513C201-RM	N/A	
Quant-iT RiboGreen RNA Reagent	Thermo Fisher, R11491	2263509	N/A	
Triton X-100 10% Solution in Water	Thermo Scientific, 85111	VJ309153	N/A	
TE Buffer	Invitrogen, AM9935	01015399	N/A	

Additional notes

Standard Curve Preparation

Prepare the standard curve material using aliquotted Pfizer 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

Tube 1: 20µL Drug Product (c. 500µg/mL) + 180µL TE buffer
Tube 2: 60µL Tube 1 (c. 50µg/mL) + 240µL TE buffer - Dilution factor: 50, to be further diluted for TE and Triton X-100 measurements

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

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

5653, 5646, 32684, 32792, 33016

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)

Plate1

	1	2	3	4	5	6	7	8	9	10	11	12
A	9.345	10.153	28.396	26.927	8.967	0.356	0.372	0.431	0.377	0.349	0.403	0.354
B	169.91	168.62	151.55	163.04	63.160	0.341	0.306	0.353	0.399	0.349	0.348	0.365
C	340.64	337.88	289.00	286.87	58.481	0.351	0.380	0.343	0.430	0.410	0.405	0.393
D	695.03	686.40	697.04	569.71	58.900	0.329	0.397	0.371	0.325	0.345	0.387	0.395
E	1060.8	1050.6	870.67	861.03	297.82	0.326	0.346	0.355	0.353	0.369	0.414	0.388
F	1364.4	1419.1	1097.5	1142.6	293.24	0.306	0.344	0.335	0.379	0.360	0.391	0.401
G	1744.7	1754.0	1414.4	1788.6	291.89	0.326	0.339	0.370	0.387	0.401	0.398	0.350
H	2054.0	2085.6	1863.3	1841.5	9.045	0.343	0.342	0.382	0.363	0.385	0.337	0.330

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 : 4:56 PM
19/07/2021

Mean Temperature : 23.4 °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

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	= 0.981	**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 = [redacted] ng/mL = [redacted] **mg/mL**

Encapsulation = [redacted] %

Sample 2: **INVALID**

Free RNA = [redacted] ng/mL

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

Encapsulation = %

Sample 3: **INVALID**

Free RNA = [redacted] ng/mL

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

Encapsulation = %

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:

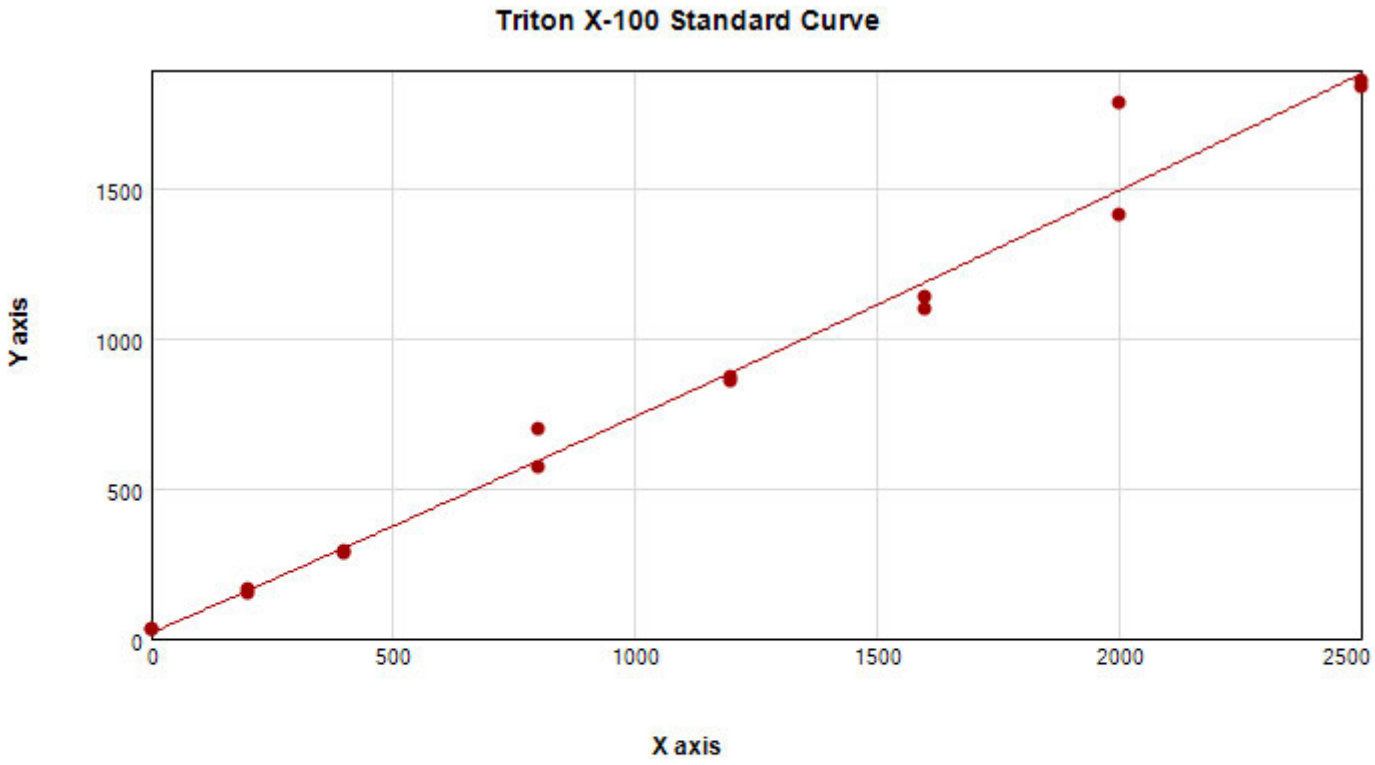
Open the active copy of Softmax Pro template "Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence.spr".

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.

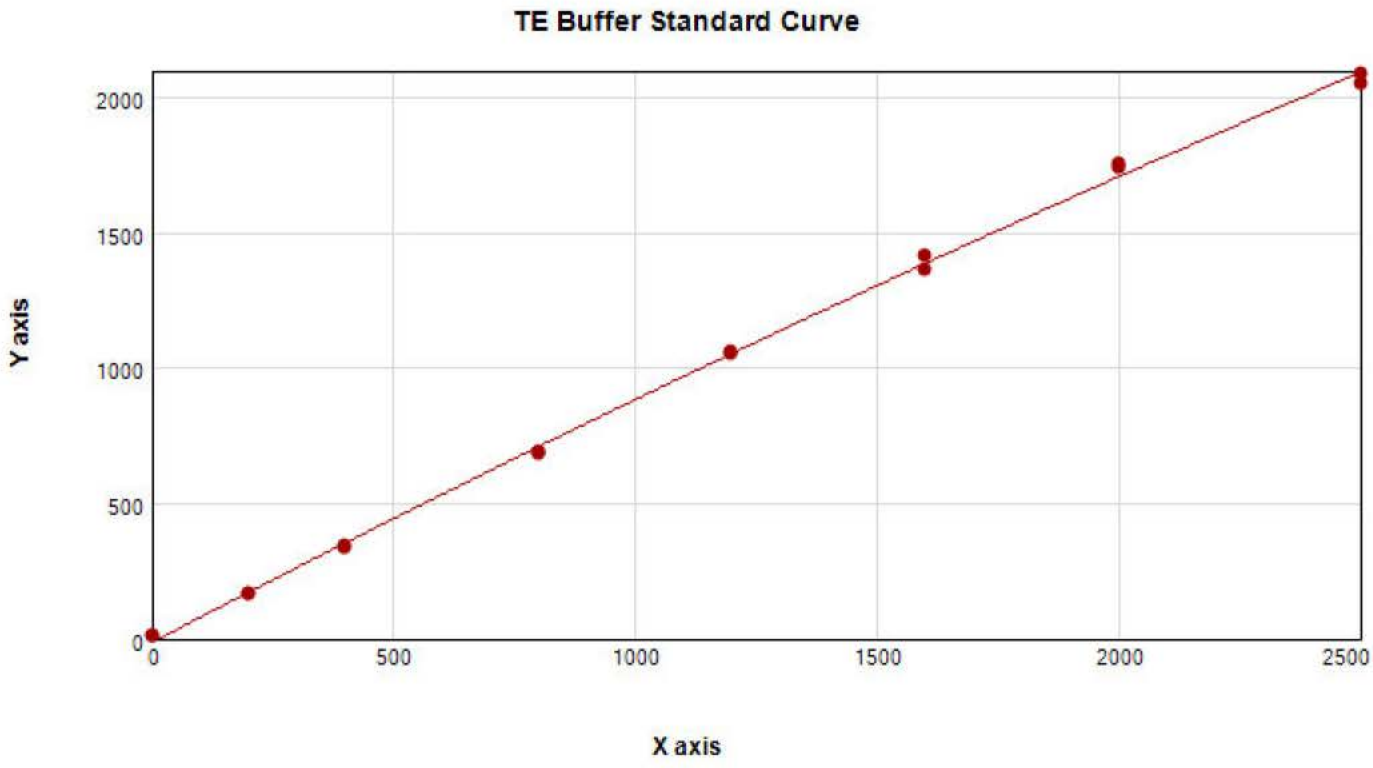


● **Plot2** (Standard Curve, Triton X-100 : Values vs Concentr...)

Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 $R^2 = 0.981$	A	19.08	49.71	[-88.31, 126.5]
	B	0.707	0.104	[0.483, 0.930]
	C	1.64e-5	4.12e-5	[-7.26e-5, 1.05e-4]



Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1 R^2 = 0.999	A	-12.55	13.63	[-41.99, 16.89]
	B	0.935	0.028	[0.873, 0.996]
	C	-3.69e-5	1.13e-5	[-6.12e-5, -1.25e-5]

Sample 1, Triton X-100 Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTX1 (ng/mL)	Std.Dev.	CV%
01	E5 F5 G5	[redacted]					4.322	1.119

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 C5 D5	[redacted]					2.787	3.571

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	[redacted]					0.016	0.062
	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	[redacted]					0.012	0.086
	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	[redacted]					0.005	0.019
	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	[redacted]					0.052	0.375
	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...
01	A3	0.000	28.396	27.662	1.039	3.755	1	0	27.662	0.000	0.000	0.000	0.000
	A4		26.927										
02	B3	200.000	151.547	157.293	8.127	5.167	0	0	0.000	157.293	0.000	0.000	0.000
	B4		163.040										
03	C3	400.000	288.997	287.933	1.505	0.523	0	0	0.000	0.000	287.933	0.000	0.000
	C4		286.869										
04	D3	800.000	697.041	633.376	90.036	14.2...	0	0	0.000	0.000	0.000	633.376	0.000
	D4		569.711										
05	E3	1200.000	870.673	865.853	6.817	0.787	0	0	0.000	0.000	0.000	0.000	865.853
	E4		861.033										
06	F3	1600.000	1097....	1120.051	31.894	2.848	0	0	0.000	0.000	0.000	0.000	0.000
	F4		1142....										
07	G3	2000.000	1414....	1601.501	264.548	16.5...	0	0	0.000	0.000	0.000	0.000	0.000
	G4		1788....										
08	H3	2500.000	1863....	1852.392	15.426	0.833	0	0	0.000	0.000	0.000	0.000	0.000
	H4		1841....										

1600ng...	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
0.000	0.000	0.000
1120.051	0.000	0.000
0.000	1601.5...	0.000
0.000	0.000	1852.392

Validation Check Sum by RSD = 0 MaxTXStdCve = 1852.392 MinTXStdCve = 27.662 Monotonic increase = VALID

Standard Curve, TE Buffer

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	0ngVal...	200...	400ngV...	800ng...	1200ng...	1600n...	2000n...	2500n...
01	A1	0.000	9.345	9.749	0.571	5.861	1	0	9.749	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	A2		10.153													
02	B1	200.000	169.906	169.263	0.909	0.537	0	0	0.000	169....	0.000	0.000	0.000	0.000	0.000	0.000
	B2		168.620													
03	C1	400.000	340.643	339.262	1.952	0.575	0	0	0.000	0.000	339.262	0.000	0.000	0.000	0.000	0.000
	C2		337.882													
04	D1	800.000	695.028	690.713	6.103	0.884	0	0	0.000	0.000	0.000	690.713	0.000	0.000	0.000	0.000
	D2		686.397													
05	E1	1200.000	1060....	1055.703	7.157	0.678	0	0	0.000	0.000	0.000	0.000	1055.703	0.000	0.000	0.000
	E2		1050....													
06	F1	1600.000	1364....	1391.733	38.644	2.777	0	0	0.000	0.000	0.000	0.000	0.000	1391....	0.000	0.000
	F2		1419....													
07	G1	2000.000	1744....	1749.347	6.552	0.375	0	0	0.000	0.000	0.000	0.000	0.000	0.000	1749....	0.000
	G2		1753....													
08	H1	2500.000	2053....	2069.794	22.403	1.082	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2069.7...
	H2		2085....													

Validation Check Sum by RSD = 0 MaxTEStdCve = 2069.794 MinTEStdCve = 9.749 Monotonic Increase = VALID