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**	25/10/2021
Checked by	[redacted]	**TRIM link to assay data file**	el://D21-3255276?db=A7&open

Results Summary

Sample Number	LIMS#	Total RNA	% Encapsulation
1	2110003737	[redacted]	[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	9.106	9.272	25.318	24.953	8.343	0.325	0.339	0.398	0.385	0.399	0.316	0.360
B	185.75	192.56	161.67	162.81	89.555	0.319	0.356	0.384	0.386	0.384	0.419	0.323
C	370.30	382.45	306.96	304.17	101.83	0.355	0.408	0.324	0.395	0.396	0.402	0.390
D	725.04	749.49	591.82	593.06	96.925	0.347	0.394	0.351	0.386	0.351	0.392	0.372
E	1130.3	1168.5	920.64	929.47	328.97	0.330	0.351	0.341	0.400	0.421	0.374	0.413
F	1457.3	1492.5	1193.8	1199.1	322.93	0.304	0.354	0.368	0.372	0.352	0.419	0.356
G	1779.8	1825.6	1469.3	1481.0	341.30	0.379	0.383	0.331	0.385	0.405	0.396	0.407
H	2458.4	2548.0	1923.9	1936.3	8.839	0.353	0.361	0.408	0.379	0.394	0.351	0.387

Reduction Settings

Wavelength Combination : !Lm1

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 : 4:10 PM
25/10/2021

Mean Temperature : 25 °C

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	= 0.996	**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 = [redacted] 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:

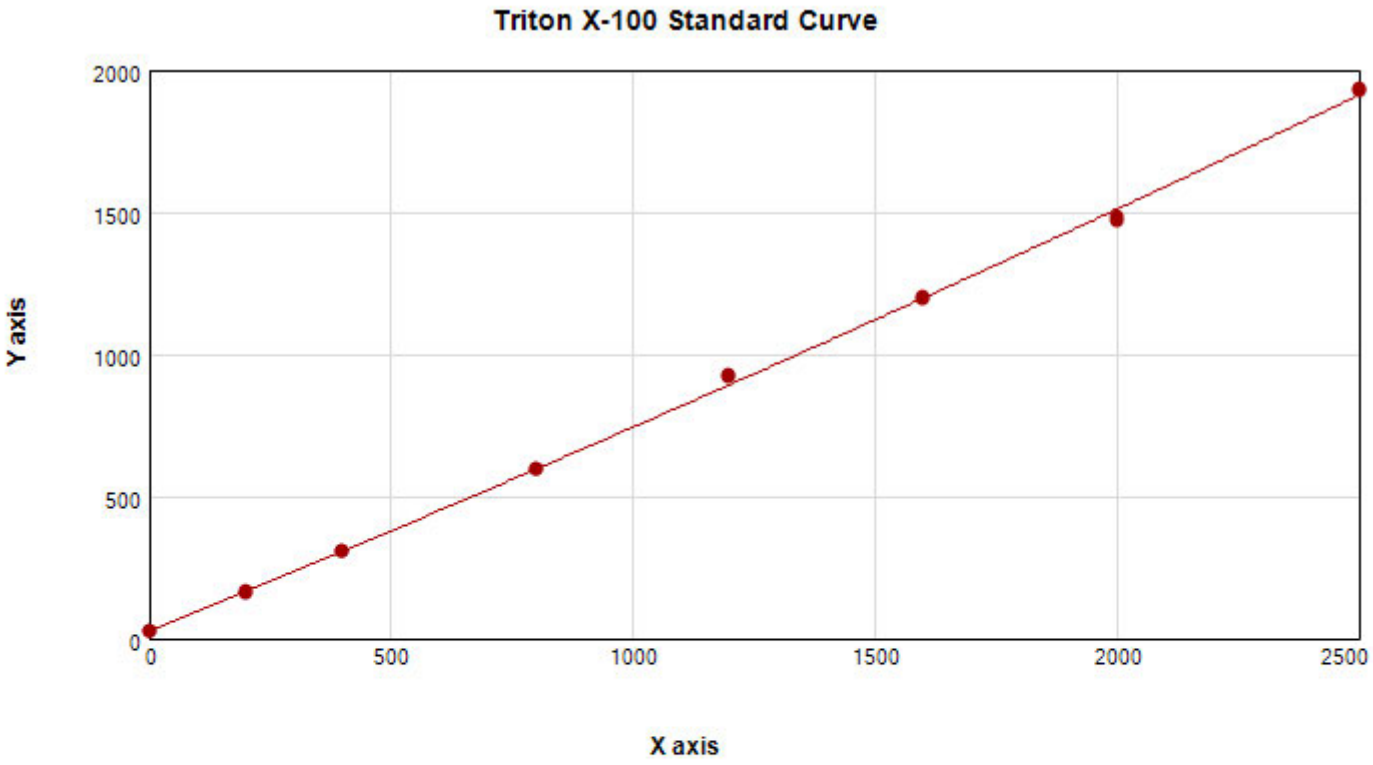
Open the active copy of Softmax Pro template "Quantitation of Total and Percent Encapsulated RNA in PF-07302048 Lipid Nanoparticles by RiboGreen Fluorescence.spr".

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.

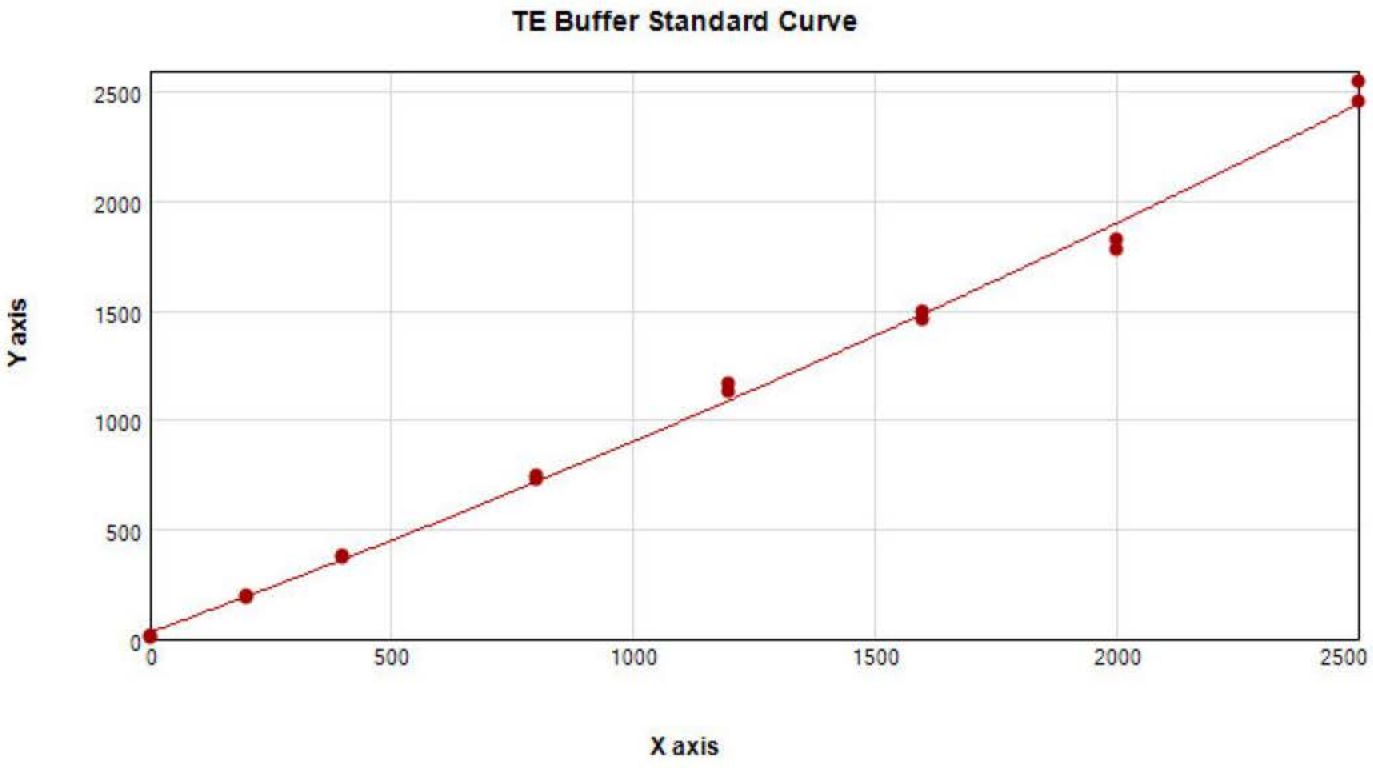


● **Plot2** (Standard Curve, Triton X-100 : Values vs Concentr...)

Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 R^2 = 0.999	A	25.66	10.64	[2.675, 48.64]
	B	0.695	0.022	[0.647, 0.743]
	C	2.40e-5	8.81e-6	[4.99e-6, 4.31e-5]



Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1 R^2 = 0.996	A	30.27	29.27	[-32.96, 93.50]
	B	0.811	0.061	[0.679, 0.943]
	C	6.26e-5	2.42e-5	[1.02e-5, 1.15e-4]

Sample 1, Triton X-100 Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTX1 (ng/mL)	Std.Dev.	CV%
01	E5 F5 G5	[redacted]					13.087	3.022

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]					7.523	9.326

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]					0.055	0.151
	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]					0.023	0.063
	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]					0.026	0.070
	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]					0.033	0.090
	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	25.318	25.136	0.258	1.027	1	0	25.136	0.000	0.000	0.000	0.000	0.000
	A4		24.953											
02	B3	200.000	161.669	162.242	0.810	0.499	0	0	0.000	162.242	0.000	0.000	0.000	0.000
	B4		162.814											
03	C3	400.000	306.962	305.565	1.975	0.646	0	0	0.000	0.000	305.565	0.000	0.000	0.000
	C4		304.169											
04	D3	800.000	591.819	592.440	0.878	0.148	0	0	0.000	0.000	0.000	592.440	0.000	0.000
	D4		593.061											
05	E3	1200.000	920.639	925.054	6.244	0.675	0	0	0.000	0.000	0.000	0.000	925.054	0.000
	E4		929.469											
06	F3	1600.000	1193....	1196.421	3.755	0.314	0	0	0.000	0.000	0.000	0.000	0.000	1196.421
	F4		1199....											
07	G3	2000.000	1469....	1475.125	8.283	0.562	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G4		1480....											
08	H3	2500.000	1923....	1930.097	8.702	0.451	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H4		1936....											

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
1475.1...	0.000
0.000	1930.097

Validation Check Sum by RSD = 0 MaxTXStdCve = 1930.097 MinTXStdCve = 25.136 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	9.106	9.189	0.117	1.277	1	0	9.189	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	A2		9.272													
02	B1	200.000	185.749	189.153	4.813	2.545	0	0	0.000	189....	0.000	0.000	0.000	0.000	0.000	0.000
	B2		192.556													
03	C1	400.000	370.298	376.373	8.591	2.283	0	0	0.000	0.000	376.373	0.000	0.000	0.000	0.000	0.000
	C2		382.448													
04	D1	800.000	725.043	737.266	17.286	2.345	0	0	0.000	0.000	0.000	737.266	0.000	0.000	0.000	0.000
	D2		749.489													
05	E1	1200.000	1130....	1149.389	26.988	2.348	0	0	0.000	0.000	0.000	0.000	1149.389	0.000	0.000	0.000
	E2		1168....													
06	F1	1600.000	1457....	1474.905	24.829	1.683	0	0	0.000	0.000	0.000	0.000	0.000	1474....	0.000	0.000
	F2		1492....													
07	G1	2000.000	1779....	1802.684	32.427	1.799	0	0	0.000	0.000	0.000	0.000	0.000	0.000	1802....	0.000
	G2		1825....													
08	H1	2500.000	2458....	2503.206	63.332	2.530	0	0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2503.2...
	H2		2547....													

Validation Check Sum by RSD = 0 MaxTEStdCve = 2503.206 MinTEStdCve = 9.189 Monotonic Increase = VALID