



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	
Authorised	
Date issued	10 August 2021
Revision #	3

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

Operator		Date of assay	18/11/2021
Checked by		TRIM link to assay data file	el://D21-3347963?db=A7&open

Results Summary

Sample Number	LIMS#	Total RNA	% Encapsulation
1	2111004115	mg/mL	
2	2111004218	mg/mL	
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

Record Details	PCR - Form - Quantitation of Total and Percent Encapsulated RNA in Lipid Nanoparticles by RiboGreen Fluorescence Worksheet		
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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, 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)

Record Details

Last Editor

Print Date

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

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Edit Date

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	Plate1											
	1	2	3	4	5	6	7	8	9	10	11	12
A	9.189	9.472	27.355	26.954	8.526	8.635	0.351	0.350	0.382	0.350	0.314	0.349
B	176.91	180.59	158.53	159.53	84.071	82.898	0.408	0.402	0.350	0.393	0.355	0.388
C	273.21	278.51	238.11	235.91	87.186	83.442	0.363	0.356	0.354	0.357	0.417	0.420
D	706.74	698.16	578.29	580.38	89.078	87.091	0.375	0.362	0.374	0.385	0.445	0.373
E	1107.8	1098.1	904.93	905.65	329.07	322.75	0.360	0.382	0.396	0.391	0.425	0.393
F	1415.6	1469.9	1193.4	1185.4	350.16	332.15	0.401	0.392	0.347	0.387	0.356	0.416
G	1688.0	1759.2	1476.8	1483.5	361.11	321.05	0.377	0.379	0.401	0.401	0.364	0.363
H	2135.1	2433.2	1927.9	1937.9	0.356	0.366	0.325	0.386	0.373	0.425	0.348	0.361

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 : 2:48 PM
18/11/2021

Mean Temperature : 24.3 °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

Assay Validity Criteria

Observed Value Validity

Coefficient of determination (R^2) for TE standard curve ≥ 0.98 = 0.992 **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) $\leq 20\%$ 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 = XXXXXXXXXX ng/mL

Total RNA = XXXXXXXXXX ng/mL = XXXXXXXXXX mg/mL

Encapsulation = XXXXXXXXXX %

Sample 2: VALID 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".

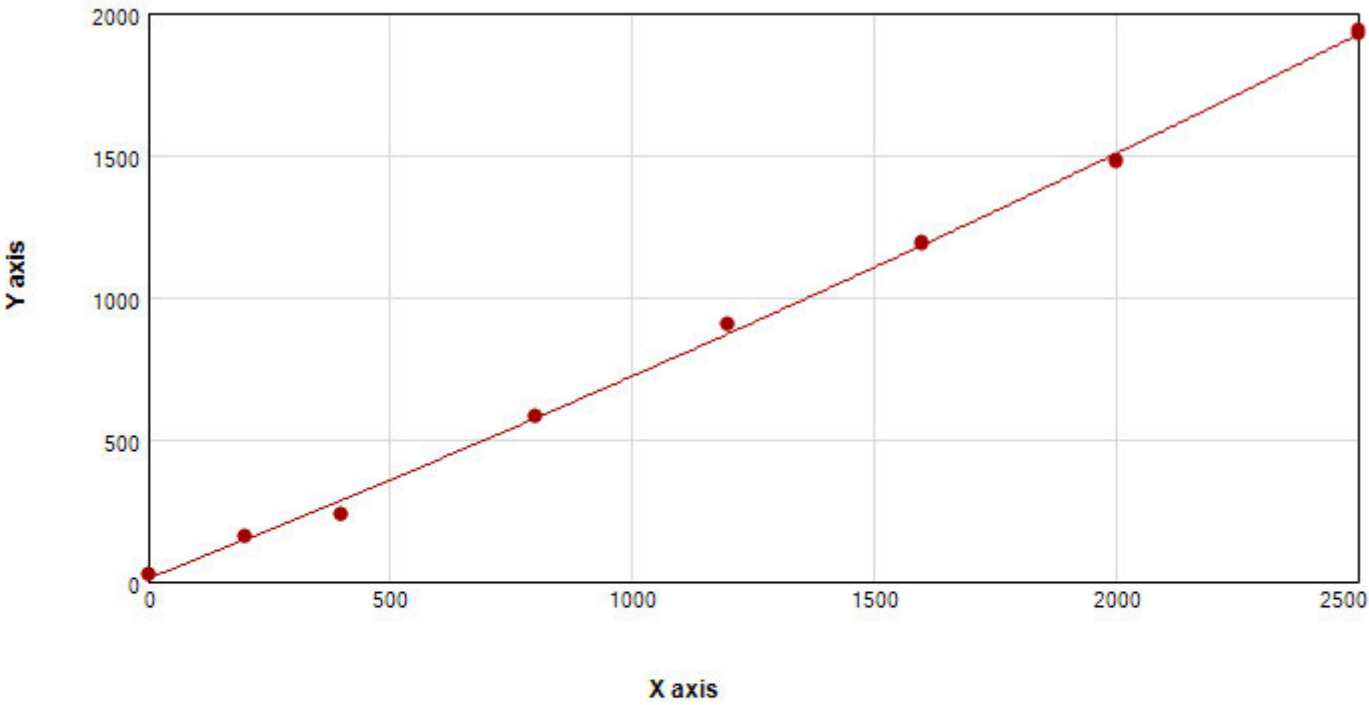
Open spreadsheet validation data set (TRIM link: D21-2134364) and copy well data from Plate 1. Paste well data into Plate 1 in the Softmax Pro Template undergonig validation.

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

Triton X-100 Standard Curve



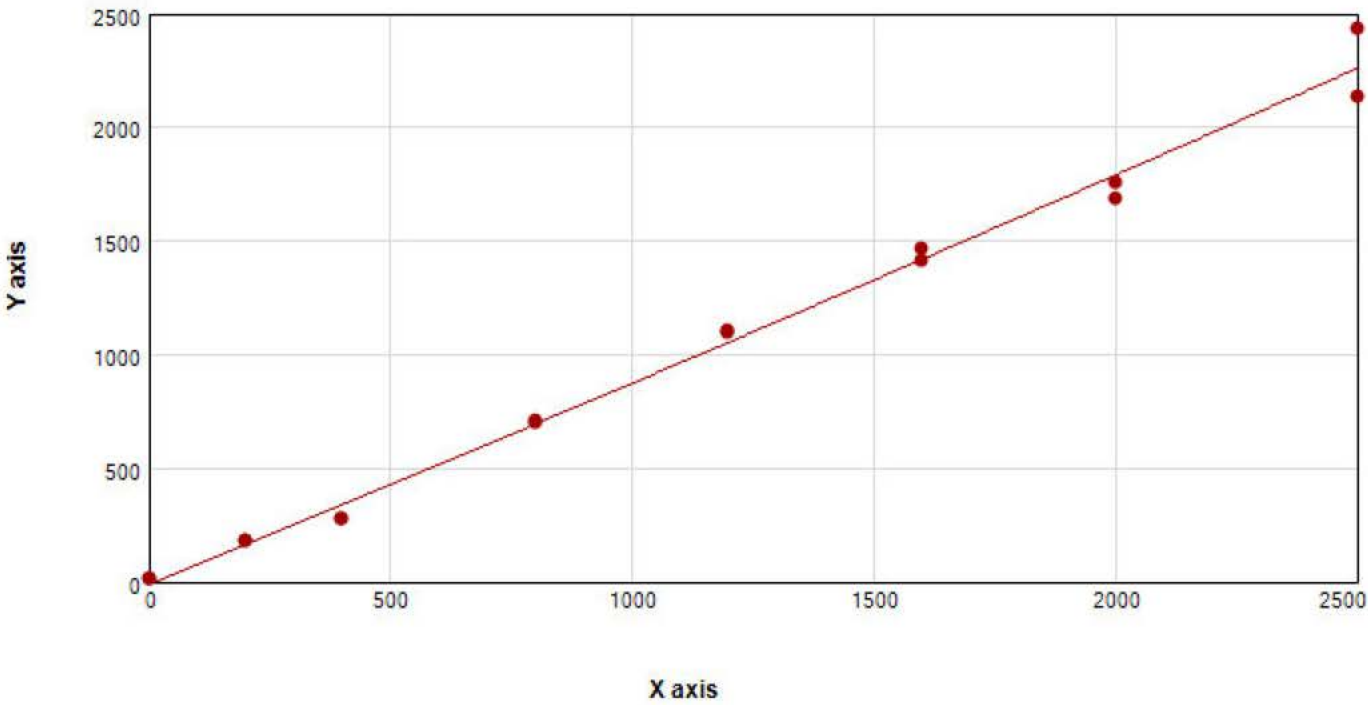
● Plot2 (Standard Curve, Triton X-100: Values vs Concentr...)

Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 $R^2 = 0.999$	A	11.41	14.06	[-18.96, 41.79]
	B	0.675	0.029	[0.612, 0.739]
	C	3.60e-5	1.16e-5	[1.09e-5, 6.12e-5]

TE Buffer Standard Curve



● Plot1 (Standard Curve, TE Buffer: Values vs Concentr...)

Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot1 $R^2 = 0.992$	A	-8.906	39.17	[-93.53, 75.72]
	B	0.868	0.082	[0.692, 1.044]
	C	1.59e-5	3.24e-5	[-5.41e-5, 8.60e-5]

Sample 1, Triton X-100 Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTX1 (ng/mL)	Std.Dev.	CV%
01	E5						22.934	4.737
	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						2.902	2.637
	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						8.441	1.860
	F6							
	G6							

Validity by RSD = VALID

Validity by range of standard curve = VALID

Sample 2, TE Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTE2 (ng/mL)	Std.Dev.	CV%
01	B6						2.617	2.437
	C6							
	D6							

Validity by RSD = VALID

Validity by range of standard curve = VALID

Sample 3, Triton X-100 Buffer

Sample	Well	Values	Mean Values	Result	MeanResult	ConcTX3 (ng/mL)	Std.Dev.	CV%
01	E7						0.031	0.187
	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						0.027	0.251
	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	27.355	27.154	0.284	1.044	1	0	27.154	0.000	0.000	0.000	0.000	0.000
	A4		26.954											
02	B3	200.000	158.534	159.034	0.707	0.445	0	0	0.000	159.034	0.000	0.000	0.000	0.000
	B4		159.534											
03	C3	400.000	238.113	237.012	1.558	0.657	0	0	0.000	0.000	237.012	0.000	0.000	0.000
	C4		235.910											
04	D3	800.000	578.289	579.334	1.478	0.255	0	0	0.000	0.000	0.000	579.334	0.000	0.000
	D4		580.379											
05	E3	1200.000	904.930	905.293	0.513	0.057	0	0	0.000	0.000	0.000	0.000	905.293	0.000
	E4		905.655											
06	F3	1600.000	1193....	1189.394	5.617	0.472	0	0	0.000	0.000	0.000	0.000	0.000	1189.394
	F4		1185....											
07	G3	2000.000	1476....	1480.122	4.765	0.322	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G4		1483....											
08	H3	2500.000	1927....	1932.891	7.056	0.365	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H4		1937....											

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
1480.1...	0.000
0.000	1932.891

Validation Check Sum by RSD = 0 MaxTXStdCve = 1932.891 MinTXStdCve = 27.154 Monotonic increase = VALID

Standard Curve, TE Buffer

Sample	Well	Concentration	Values	MeanValue	Std.Dev.	CV%	Is0ng	ValRSD	OngVal...	200...	400ngV...	800ng...	1200ng...	1600n...
01	A1	0.000	9.189	9.331	0.200	2.145	1	0	9.331	0.000	0.000	0.000	0.000	0.000
	A2		9.472											
02	B1	200.000	176.908	178.747	2.600	1.455	0	0	0.000	178....	0.000	0.000	0.000	0.000
	B2		180.585											
03	C1	400.000	273.211	275.860	3.746	1.358	0	0	0.000	0.000	275.860	0.000	0.000	0.000
	C2		278.509											
04	D1	800.000	706.741	702.448	6.071	0.864	0	0	0.000	0.000	0.000	702.448	0.000	0.000
	D2		698.156											
05	E1	1200.000	1107....	1102.953	6.910	0.626	0	0	0.000	0.000	0.000	0.000	1102.953	0.000
	E2		1098....											
06	F1	1600.000	1415....	1442.738	38.367	2.659	0	0	0.000	0.000	0.000	0.000	0.000	1442....
	F2		1469....											
07	G1	2000.000	1688....	1723.613	50.295	2.918	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G2		1759....											
08	H1	2500.000	2135....	2284.150	210.748	9.227	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H2		2433....											

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
1723....	0.000
0.000	2284.1...

Validation Check Sum by RSD = 0 MaxTEStdCve = 2284.150 MinTEStdCve = 9.331 Monotonic Increase = VALID