



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	
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Revision #	3

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

Operator		Date of assay	01/11/2021
Checked by		TRIM link to assay data file	el://D21-3277983?db=A7&open

Results Summary

Sample Number	LIMS#	Total RNA	% Encapsulation
1	2110003905	mg/mL	
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

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

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		
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	Plate1											
	1	2	3	4	5	6	7	8	9	10	11	12
A	9.346	9.856	26.222	26.824	8.649	0.383	0.316	0.346	0.354	0.383	0.397	0.376
B	190.38	196.90	163.99	168.86	100.17	0.335	0.357	0.364	0.412	0.370	0.407	0.370
C	376.78	391.55	307.76	315.47	92.986	0.393	0.389	0.405	0.386	0.356	0.378	0.400
D	747.33	779.44	613.25	621.49	101.15	0.350	0.302	0.327	0.361	0.369	0.412	0.360
E	1157.9	1212.2	944.46	959.95	325.07	0.326	0.344	0.345	0.399	0.344	0.388	0.393
F	1513.9	1585.7	1234.5	1255.1	346.73	0.347	0.359	0.387	0.374	0.344	0.377	0.401
G	1850.7	1937.3	1517.3	1556.0	345.42	0.371	0.381	0.375	0.360	0.357	0.423	0.417
H	2494.5	2616.6	1930.5	1946.4	8.873	0.330	0.323	0.442	0.357	0.361	0.357	0.371

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 : 11:04 AM
1/11/2021

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 = 0.997 **VALID**Coefficient of determination (R^2) for TX standard curve ≥ 0.98 = 1.000 **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 = [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".

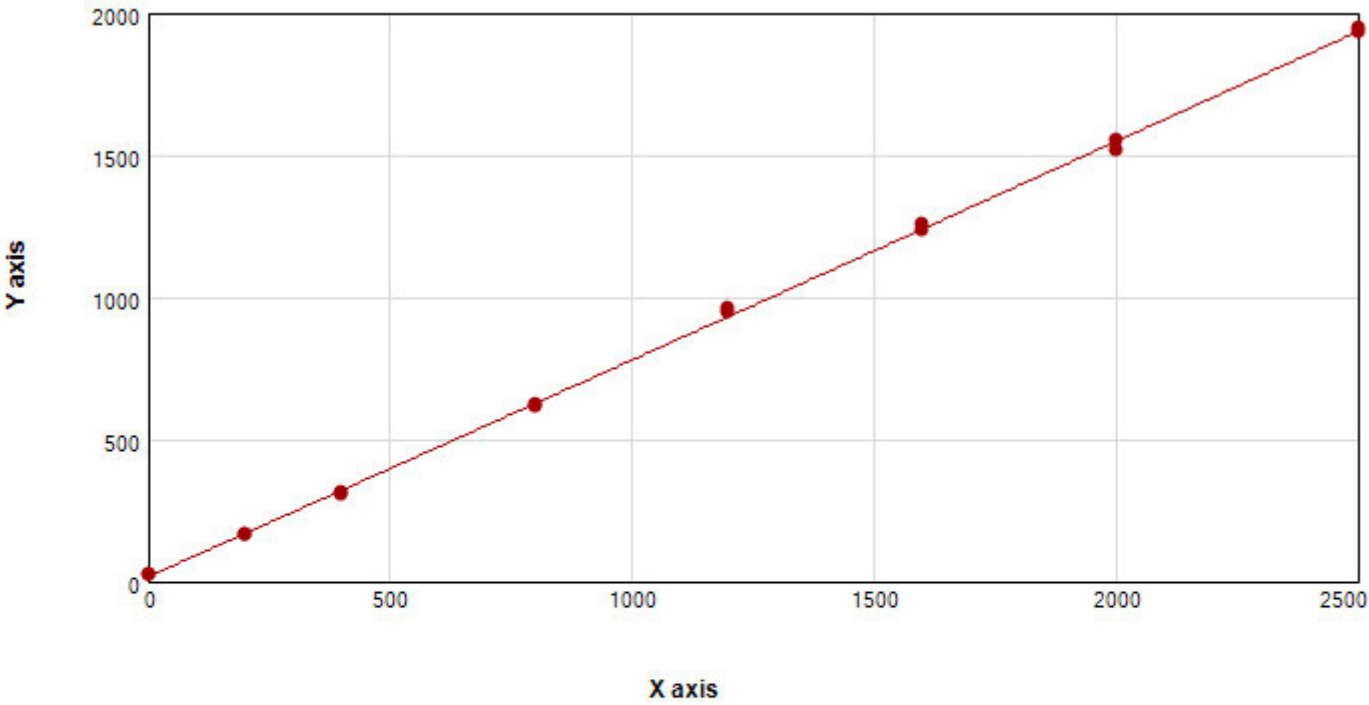
Open spreadsheet validation data set (TRIM link: D21-2134364) and copy well data from Plate 1. Paste well data into Plate 1 in the Softmax Pro Template undergonig validation.

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



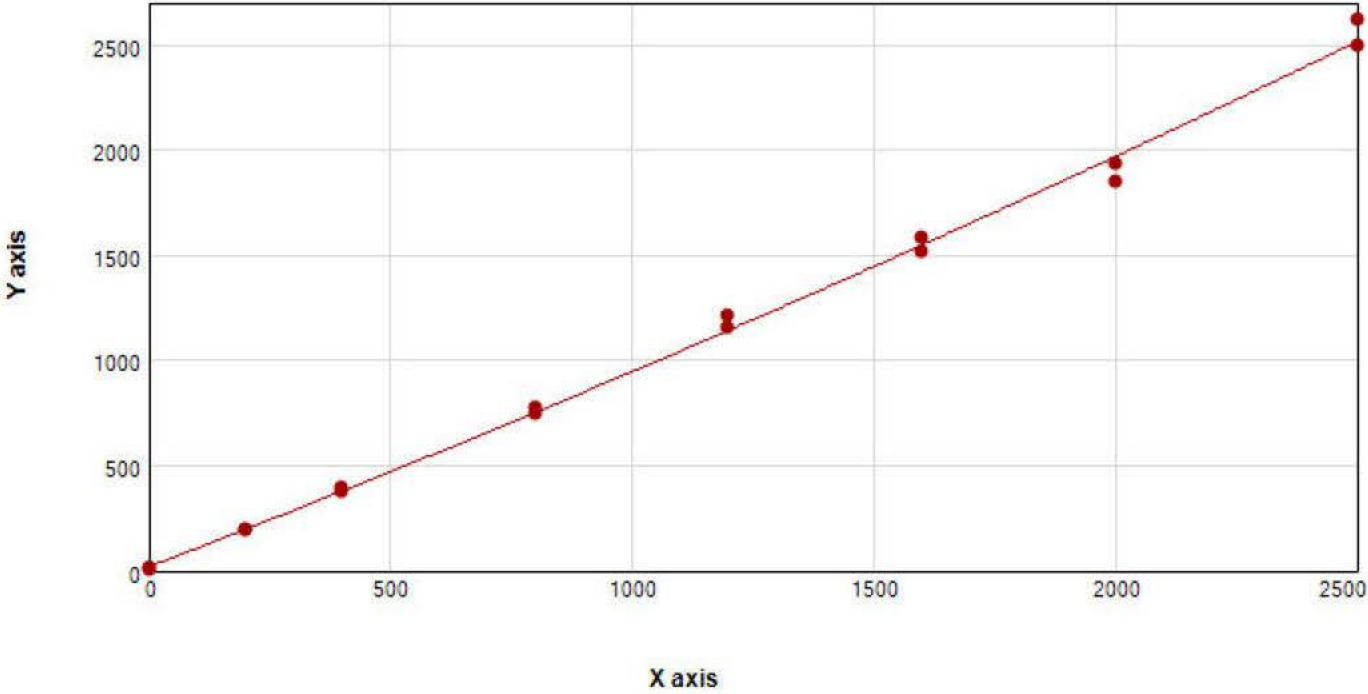
● Plot2 (Standard Curve, Triton X-100: Values vs Concentr...)

Curve Fit Results ▲

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

	Parameter	Estimated Value	Std. Error	Confidence Interval
Plot2 $R^2 = 1.000$	A	17.41	7.799	[0.559, 34.25]
	B	0.760	0.016	[0.725, 0.795]
	C	2.99e-6	6.46e-6	[-1.10e-5, 1.69e-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 $R^2 = 0.997$	A	20.49	26.69	[-37.18, 78.15]
	B	0.880	0.056	[0.760, 1.000]
	C	4.76e-5	2.21e-5	[-1.68e-7, 9.53e-5]

Sample 1, Triton X-100 Buffer

Sample	Well	Values	MeanValues	Result	MeanResult	ConcTX1 (ng/mL)	Std.Dev.	CV%
01	E5						15.933	3.769
	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						5.018	5.715
	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						0.030	0.132
	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						0.034	0.150
	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						0.024	0.109
	F7							
	G7							

Validity 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						0.050	0.219
	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	26.222	26.523	0.426	1.605	1	0	26.523	0.000	0.000	0.000	0.000	0.000
	A4		26.824											
02	B3	200.000	163.985	166.423	3.448	2.072	0	0	0.000	166.423	0.000	0.000	0.000	0.000
	B4		168.861											
03	C3	400.000	307.755	311.613	5.457	1.751	0	0	0.000	0.000	311.613	0.000	0.000	0.000
	C4		315.472											
04	D3	800.000	613.249	617.369	5.827	0.944	0	0	0.000	0.000	0.000	617.369	0.000	0.000
	D4		621.489											
05	E3	1200.000	944.461	952.206	10.953	1.150	0	0	0.000	0.000	0.000	0.000	952.206	0.000
	E4		959.951											
06	F3	1600.000	1234....	1244.814	14.565	1.170	0	0	0.000	0.000	0.000	0.000	0.000	1244.814
	F4		1255....											
07	G3	2000.000	1517....	1536.639	27.315	1.778	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G4		1555....											
08	H3	2500.000	1930....	1938.443	11.242	0.580	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H4		1946....											

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
1536.6...	0.000
0.000	1938.443

Validation Check Sum by RSD = 0 MaxTXStdCve = 1938.443 MinTXStdCve = 26.523 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	9.346	9.601	0.361	3.756	1	0	9.601	0.000	0.000	0.000	0.000	0.000
	A2		9.856											
02	B1	200.000	190.385	193.642	4.605	2.378	0	0	0.000	193....	0.000	0.000	0.000	0.000
	B2		196.898											
03	C1	400.000	376.778	384.164	10.446	2.719	0	0	0.000	0.000	384.164	0.000	0.000	0.000
	C2		391.551											
04	D1	800.000	747.330	763.384	22.704	2.974	0	0	0.000	0.000	0.000	763.384	0.000	0.000
	D2		779.438											
05	E1	1200.000	1157....	1185.059	38.405	3.241	0	0	0.000	0.000	0.000	0.000	1185.059	0.000
	E2		1212....											
06	F1	1600.000	1513....	1549.778	50.768	3.276	0	0	0.000	0.000	0.000	0.000	0.000	1549....
	F2		1585....											
07	G1	2000.000	1850....	1893.993	61.182	3.230	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	G2		1937....											
08	H1	2500.000	2494....	2555.591	86.335	3.378	0	0	0.000	0.000	0.000	0.000	0.000	0.000
	H2		2616....											

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
1893....	0.000
0.000	2555.5...

Validation Check Sum by RSD = 0 MaxTEStdCve = 2555.591 MinTEStdCve = 9.601 Monotonic Increase = VALID