moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

1. Introduction

This report presents the method validation results of test method SOP-0937, mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System, for mRNA-1273 Lipid Nanoparticle (LNP) and Drug Product (DP). SOP-0937 is categorized as a content/potency assay and was previously qualified as reported in MQR-0205. The validation was performed at Moderna's Quality Control Laboratory following method validation protocol QC-MVP-0019 v1.0 and is based on the ICH Q2(R1) guidance "Validation of Analytical Procedures: Text and Methodology".

The principle of this assay is to assess the ability of mRNA-1273 LNP/DP to translate a polypeptide of expected molecular weight using a Cell-Free In-Vitro Translation System and gel electrophoresis as a preliminary potency assay. This method uses a [redacted] to translate encoded proteins from mRNA extracted from mRNA LNPs/DPs, using a [redacted]

Method SOP-0937 was validated according to QC-MVP-0019 v1.0, using one lot of mRNA-1273 LNP (5006820001) and two lots of mRNA-1273 DP (6006820001 and 6006920001).The validation results provide an assessment of method Specificity, Accuracy, and Precision. All parameters assessed met the acceptance criteria outlined in QC-MVP-0019. Test method SOP-0937 is considered suitable and is validated for testing mRNA-1273 LNP and DP.

2. Referenced Documents

Document #	Title
ICH Q2(R1)	Validation of Analytical Procedures: Text and Methodology
FRM-0043	Read and Understand Training Form
FRM-0679	In-Vitro Translation Assay Worksheet – mRNA-1273, SOP-0937
MQR-0205	Qualification Report of SOP-0937: mRNA-1273
SOP-0937	mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System
QC-MVP-0019	Method Validation Protocol of SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

3. Responsibilities

Department/ Functional Area	Responsibility
Quality Control	• Authors, reviews and approves validation protocols and reports. • Executes, reviews and approves executed data packages and data summaries. • Authors validation summary reports.
Quality Assurance	• Reviews and approves validation protocols, data summaries, and reports. • Ensures that validation documents are in alignment with Moderna policies and regulatory requirements.

4. Definitions

Term	Definition
DP	Drug Product
DS	Drug Substance
GMP	Good Manufacturing Practices
LNP	Lipid Nanoparticle
QC	Quality Control

5. Documentation

5.1. All documentation, execution, and review of the work performed for this study was conducted under current Good Manufacturing Practices (cGMP) as required by Moderna standard operating procedures.

5.2. **SOP-0937 v2.0** was followed for this testing. Assay information was documented on Assay Preference Worksheet (APW) **FRM-0679 v3.0.**

5.3. One discrepancy was identified during the execution of this validation protocol (see **Section 9.**). A different lot of positive control was used in execution of the validation. No impact.

5.4. All relevant data collected during validation and formulae used for calculating validation characteristics were peer reviewed and included as attachments to this validation report.

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

6. Materials and Equipment

SOP-0937 v2.0 was followed for the validation testing.

6.1. Test Articles

6.1.1. Test Samples

Sample Description	Lot/Batch	RNA Concentration (mg/mL)	Expected Molecular Weight (kDa)	Summary of Analysis Document
mRNA-1273 LNP[1]	5006820001	[redacted]	[redacted]	COA-0447
mRNA-1273 DP[2]	6006820001			COA-0448
mRNA-1273 DP[2]	6006920001			COA-0449

[1] mRNA-1273 Lipid Nanoparticle (LNP) is prepared as a liquid solution with a target mRNA concentration of [redacted] mg/mL in 16.7 mM sodium acetate, 20 mM Tris, 87 g/L Sucrose, pH 7.5 buffer.

[2] mRNA-1273 Drug Product (DP) is prepared as a liquid solution with a target mRNA concentration of approximately [redacted] mg/mL in 4.3 mM sodium acetate, 20 mM Tris, 87 g/L sucrose, pH 7.5 buffer.

6.1.2. Molecular Weight (MW) Ladder Standard

Reference Standard	Supplier	Catalog Number
Precision Plus Protein All Blue Standard	Bio-Rad	1610373

6.1.3. Negative Control

Replace mRNA in translation reaction with nuclease-free water.

6.1.4. Positive Control (mRNA Product Intermediate)

Sample Type	Sample/Lot	Expected Molecular Weight (kDa)	Concentration (mg/mL)	Summary of Analysis Document
Positive Control	CX-024414/ MTDS20002[1]	[redacted]		DSAD-SOA-0254

[1] Lot MTDS20002 was used in lieu of lot AMPDS200006. See Discrepancy 1 in Section 9.

6.2. Materials and Equipment

6.2.1. Refer to the Materials and Equipment Section of SOP-0937 v2.0.

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

7. Validation Results

7.1. Summary of Validation Results

Parameter	Acceptance Criteria	Results	Pass/Fail
Specificity	The expected MW band observed in the positive control must have correlative bands observed in the mRNA-1273 lanes, with the mean MW band within [redacted] of the expected MW. The negative control must not have bands that correlate with the expected MW. Expected MW (kDa): [redacted] MW [redacted] Acceptance Range (kDa): [redacted] **Runs 1-2: Analyst "A"** **Run 3: Analyst "B"**	The expected bands were present and within [redacted] of the expected MW the positive control CX-024414 and three mRNA-1273 lots. No bands correlating to the MWs of the positive control or drug product were observed in the negative controls. **(Table 1)**	**Pass**
Accuracy	The calculated MW of positive controls and the calculated mean MW for the mRNA-1273 lots must be within [redacted] of the expected MW. Expected MW (kDa): [redacted] MW [redacted] Acceptance Range (kDa): [redacted] **Runs 1-2: Analyst "A"** **Run 3: Analyst "B"**	The observed MW of the positive controls CX-024414 was within [redacted] of the expected MW with a maximum difference of [redacted] The observed mean MW for the three mRNA-1273 was within [redacted] with a maximum difference of [redacted] **(Table 1)**	**Pass**
Precision (Repeatability)	The %RSD (n=6) of the mean observed MW of each mRNA-1273 lot (3), run two times by one analyst on a single day, must be [redacted] **Runs 1-2: Analyst "A"**	%RSD (n=6) = [redacted] **(Table 2)**	**Pass**
Precision (Intermediate)	The %RSD (n=6) of the mean observed MW of each mRNA-1273 lot (3), prepared by two analysts on different days, must be [redacted]. **Run 1: Analyst "A"** **Run 3: Analyst "B"**	%RSD (n=6) = [redacted] **(Table 3)**	**Pass**

moderna Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

7.2. Run Strategy

Run Number	Parameter	Analyst	Raw Data ARN Reference
1	Specificity, Accuracy, Repeatability, Intermediate Precision	A	ARN-20-00353-019
2	Specificity, Accuracy, Repeatability	A	ARN-20-00353-020
3	Specificity, Accuracy, Intermediate Precision	B	ARN-20-00353-014

7.3. Specificity

Experimental Design:

SOP-0937 was executed three times on each of three mRNA-1273 lots (**Runs 1 and 2, Analyst "A"; Run 3, Analyst "B"**):

Sample Description	Lot/Batch
mRNA-1273 LNP	5006820001
mRNA-1273 DP	6006820001
mRNA-1273 DP	6006920001

Data Analysis:

The expected band in the positive controls and mRNA-1273 reaction lanes were identified and the Mean Molecular Weight (MW) was calculated for each positive control and mRNA-1273 lot based on the Molecular Weight Ladder Standard included on the gel. The presence/absence of a MW band the size of the expected reaction in the negative control was assessed.

Acceptance Criteria:

The expected MW band observed in the positive control must have correlative bands observed in the mRNA-1273 lanes, with the mean MW band within [redacted] of the expected MW. The negative control must not have bands that correlate with the expected MW.

Expected MW (kDa): [redacted]
MW [redacted] Acceptance Range (kDa): [redacted]
Runs 1-2: Analyst "A"
Run 3: Analyst "B"

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

Specificity Results:

All specificity acceptance criteria were met. The expected band was present with a mean MW within [redacted] of the expected MW for mRNA-1273 LNP (5006820001) and the two lots of mRNA-1273 DP (6006820001 and 6006920001) in all three runs (see **Table 1**). The expected band was present with a mean MW within [redacted] of the expected MW for the drug substance positive control CX-024414 in all three runs. No band correlating to the MW of the positive control drug substance or mRNA-1273 were observed in the negative controls in all three runs.

Table 1: Specificity and Accuracy Results (Band MWs)

Run	Material (Lot)	Expected Protein MW (kDa) [1]	Observed Protein MW (kDa)	Mean Protein MW (kDa)	Difference	Band Absence/ Presence Pass/Fail	Band Size Pass/Fail
Run 1 Analyst A	Negative Control	[redacted]				**Pass**	**N/A**
	Positive Control CX-024414 (MTDS20002)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (5006820001)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (6006820001)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (6006920001)	[redacted]				**Pass**	**Pass**
Run 2 Analyst A	Negative Control	[redacted]				**Pass**	**N/A**
	Positive Control CX-024414 (MTDS20002)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (5006820001)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (6006820001)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (6006920001)	[redacted]				**Pass**	**Pass**
Run 3 Analyst B	Negative Control	[redacted]				**Pass**	**N/A**
	Positive Control CX-024414 (MTDS20002)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (5006820001)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (6006820001)	[redacted]				**Pass**	**Pass**
	mRNA-1273 (6006920001)	[redacted]				**Pass**	**Pass**

[1] mRNA-1273 MW [redacted] Acceptance Range (kDa): [redacted]

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

7.4. Accuracy

Experimental Design:

SOP-0937 was executed three times on each of three mRNA-1273 lots (**Runs 1 and 2, Analyst "A"; Run 3, Analyst "B"**):

Sample Description	Lot/Batch
mRNA-1273 LNP	5006820001
mRNA-1273 DP	6006820001
mRNA-1273 DP	6006920001

Data Analysis:

For each run, the expected bands in the positive controls and mRNA-1273 reaction lanes were identified and the mean MW of each was calculated based on the Molecular Weight Ladder Standard included on the gel.

Acceptance Criteria:

The calculated MW for observed band in positive controls and the calculated mean MW for each mRNA-1273 lot must be within [redacted] of the expected MW. Report the mean MWs of the band. **Runs 1 and 2, Analyst "A"; Run 3, Analyst "B"**

Expected MW (kDa): [redacted]

MW [redacted] Acceptance Range (kDa): [redacted]

Accuracy Results:

All accuracy acceptance criteria were met. The expected band was present and the mean MW was within [redacted] of the expected MW for mRNA-1273 LNP (5006820001) and two lots of mRNA-1273 DP (6006820001 and 6006920001) in all three runs, with a maximum observed difference of [redacted] (see **Table 1**). The expected band was present and the MW was within [redacted] of the expected MW for the drug substance positive control CX-024414 in all three runs, with a maximum observed difference of [redacted]

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

7.5. Precision (Repeatability)

Experimental Design:

SOP-0937 was executed by one analyst two times on each of three mRNA-1273 lots (**Runs 1 and 2, Analyst "A"**):

Sample Description	Lot/Batch
mRNA-1273 LNP	5006820001
mRNA-1273 DP	6006820001
mRNA-1273 DP	6006920001

Data Analysis:

For each run, expected bands in the mRNA-1273 reaction lanes were identified. The mean MW of each band was calculated based on the Molecular Weight Ladder Standard included on the gel. The %RSD of the 6 resulting observed mean MWs was determined.

Acceptance Criteria:

The %RSD (n=6) of the mean observed MW of each mRNA-1273 lot (3), run two times by one analyst on a single day, must be [redacted] **Runs 1-2: Analyst "A"**

Precision (Repeatability) Results:

The Precision (Repeatability) acceptance criteria were met. The %RSD (n=6) of the mean observed MW of each mRNA-1273 lot (3), run two times by one analyst on a single day, was [redacted] (see **Table 2**).

Table 2: Precision (Repeatability)

Run	Sample Type	Mean Protein MW (kDa)	Overall Mean Protein MW (kDa)	%RSD
Run 1 Analyst A	mRNA-1273 (5006820001)	[redacted]		
	mRNA-1273 (6006820001)			
	mRNA-1273 (6006920001)			
Run 2 Analyst A	mRNA-1273 (5006820001)			
	mRNA-1273 (6006820001)			
	mRNA-1273 (6006920001)			

7.6. Precision (Intermediate)

Experimental Design:

SOP-0937 was executed by two analysts on different days on each of three mRNA-1273 lots (**Run 1, Analyst "A"; Run 3, Analyst "B"**):

Sample Description	Lot/Batch
mRNA-1273 LNP	5006820001
mRNA-1273 DP	6006820001
mRNA-1273 DP	6006920001

Data Analysis:

For each run, expected bands in the mRNA-1273 reaction lanes were identified. The mean MW of each band was calculated based on the Molecular Weight Ladder Standard included on the gel. The %RSD of the 6 resulting observed mean MWs was determined.

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

Acceptance Criteria:

The %RSD (n=6) of the mean observed MW of each mRNA-1273 lot (3), prepared by two analysts on different days, must be [redacted]. **Run 1: Analyst "A"; Run 3: Analyst "B"**

Precision (Intermediate) Results:

The Precision (Intermediate) acceptance criteria were met. The %RSD (n=6) of the mean observed MW of each mRNA-1273 lot (3), run by two analysts on different days, was [redacted] (see **Table 3**).

Table 3: Precision (Intermediate)

Run	Sample Type	Mean Protein MW (kDa)	Overall Mean Protein MW (kDa)	%RSD
Run 1 Analyst A	mRNA-1273 (5006820001)	[redacted]		
	mRNA-1273 (6006820001)			
	mRNA-1273 (6006920001)			
Run 3 Analyst B	mRNA-1273 (5006820001)			
	mRNA-1273 (6006820001)			
	mRNA-1273 (6006920001)			

8. Conclusions

All acceptance criteria for validation parameters Specificity, Accuracy and Precision outlined in QC-MVP-0019 have been met for SOP-0937.
Analytic test method SOP-0937 is considered validated for testing mRNA-1273 LNP and DP.

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

9. Discrepancies

9.1. Discrepancy 1

9.1.1. Description
QC-MVP-0019 (Section 6.1.4.) indicated that mRNA Product Intermediate Positive Control lot AMPDS200006 would be used. Due to lack of availability of this lot, Lot MTDS200002 (DSAD-SOA-0254) of the same material was used during execution of QC-MVP-0019.

9.1.2. Impact Assessment
No impact. A comparable mRNA-1273 Product Intermediate lot was used and performed as expected in the Validation.

10. Attachments

10.1. Included in Document

10.1.1. ATTACHMENT 1: Gel Images

10.2. Electronically Attached in [redacted]

10.2.1. ARN-20-00353-019 QC-MVP-0019 Run 1

10.2.2. ARN-20-00353-020 QC-MVP-0019 Run 2

10.2.3. ARN-20-00353-014 QC-MVP-0019 Run 3

10.2.4. QC-MVR-0020 IVT Excel Calculations Spreadsheet

10.2.5. Verified Signed Copy of QC-MVR-0020 Excel Calculations

11. Revision History

Revision #	Effective Date	Change Details	Author
1.0	Refer to [redacted] Header for Effective Date	New Document	[redacted]

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

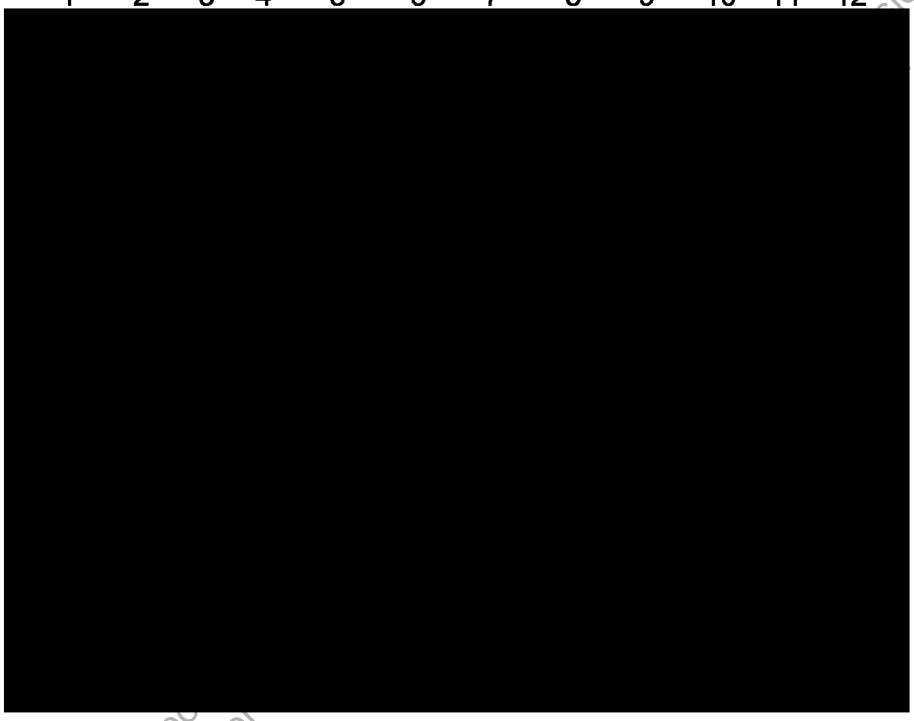
mRNA-1273 LNP, mRNA-1273 DP

ATTACHMENT 1 – Gel Images

(Page 1 of 3)

Figure 1: Run 1 Gel Image (ARN-20-000353-019)

1 2 3 4 5 6 7 8 9 10 11 12



Lane	Material
1	*Running Buffer Alone*
2	MW Ladder Standard
3	Negative Control
4	CX-024414 Positive Control
5	mRNA-1273 Lot 5006820001 (a)
6	mRNA-1273 Lot 5006820001 (b)
7	mRNA-1273 Lot 6006820001 (a)
8	mRNA-1273 Lot 6006820001 (b)
9	mRNA-1273 Lot 6006920001 (a)
10	mRNA-1273 Lot 6006920001 (b)
11	MW Ladder Standard
12	*Running Buffer Alone*

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

mRNA-1273 LNP, mRNA-1273 DP

ATTACHMENT 1 – Gel Images
(Page 2 of 3)

Figure 2: Run 2 Gel Image (ARN-20-000353-020)

1 2 3 4 5 6 7 8 9 10 11 12

[redacted]

Lane	Material
1	*Running Buffer Alone*
2	MW Ladder Standard
3	Negative Control
4	CX-024414 Positive Control
5	mRNA-1273 Lot 5006820001 (a)
6	mRNA-1273 Lot 5006820001 (b)
7	mRNA-1273 Lot 6006820001 (a)
8	mRNA-1273 Lot 6006820001 (b)
9	mRNA-1273 Lot 6006920001 (a)
10	mRNA-1273 Lot 6006920001 (b)
11	MW Ladder Standard
12	*Running Buffer Alone*

moderna | Method Validation Report

TITLE

Method Validation Report to QC-MVP-0019 for SOP-0937: mRNA-1273 Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System

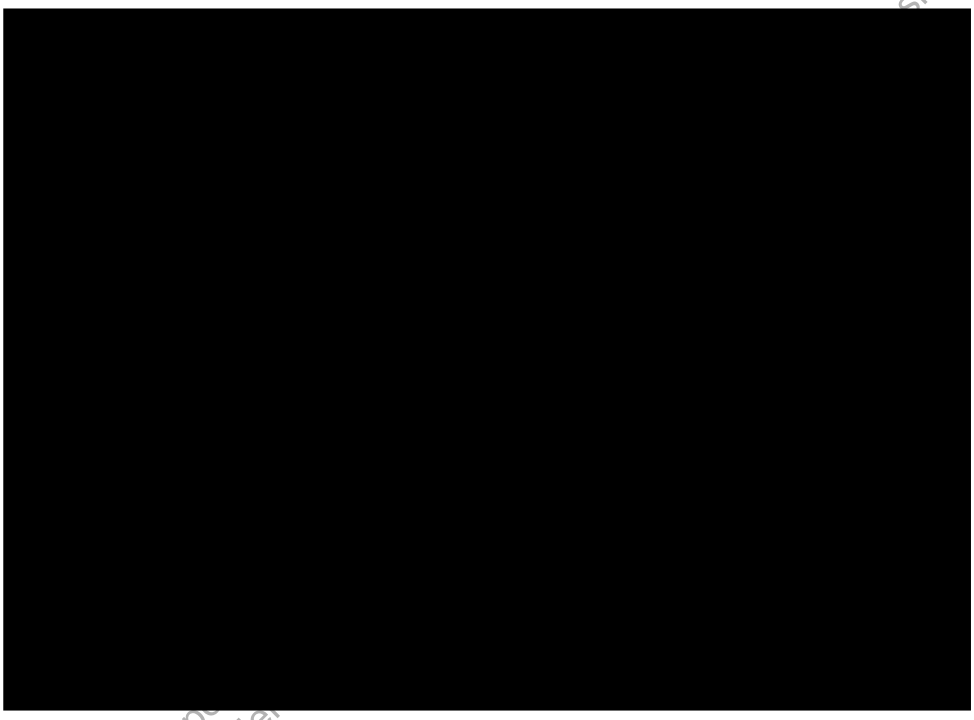
mRNA-1273 LNP, mRNA-1273 DP

ATTACHMENT 1 – Gel Images

(Page 3 of 3)

Figure 3: Run 3 Gel Image (ARN-20-000353-014)

1 2 3 4 5 6 7 8 9 10 11 12



Lane	Material
1	*Running Buffer Alone*
2	MW Ladder Standard
3	Negative Control
4	CX-024414 Positive Control
5	mRNA-1273 Lot 6006820001 (a)
6	mRNA-1273 Lot 6006820001 (b)
7	mRNA-1273 Lot 6006920001 (a)
8	mRNA-1273 Lot 6006920001 (b)
9	mRNA-1273 Lot 5006820001 (a)
10	mRNA-1273 Lot 5006820001 (b)
11	MW Ladder Standard
12	*Running Buffer Alone*

Document Approvals

Approved Date: 02 Oct 2020

Task: Approval Task Verdict: Approve	[redacted]
Task: Approval Task Verdict: Approve	[redacted]
Task: QA Approval Task Verdict: Approve	[redacted]