

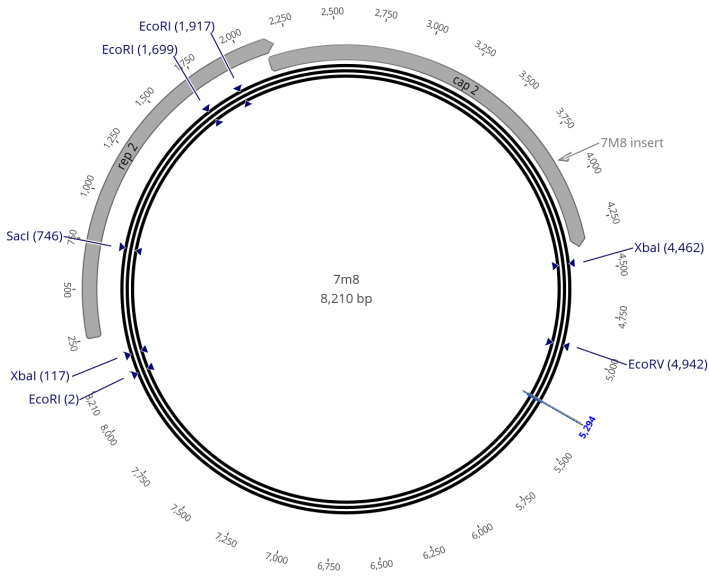
**AAV Batch #: 7m8-20241115-scCAG-GFP**

Serotype: 7m8

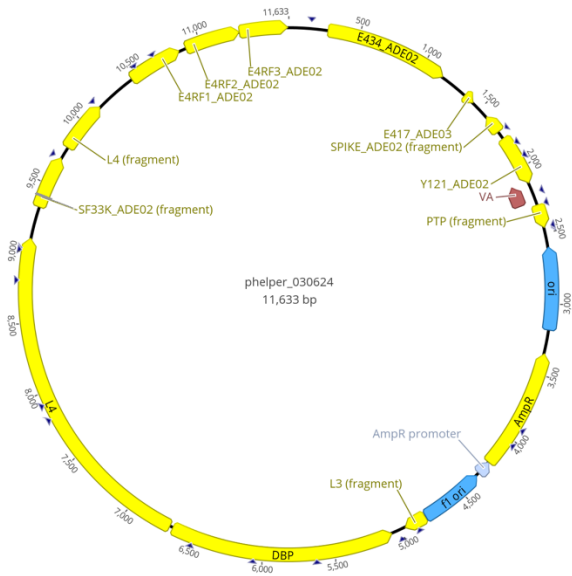
AAV construct: pAAV-scCAG-GFP

**Plasmid map:**

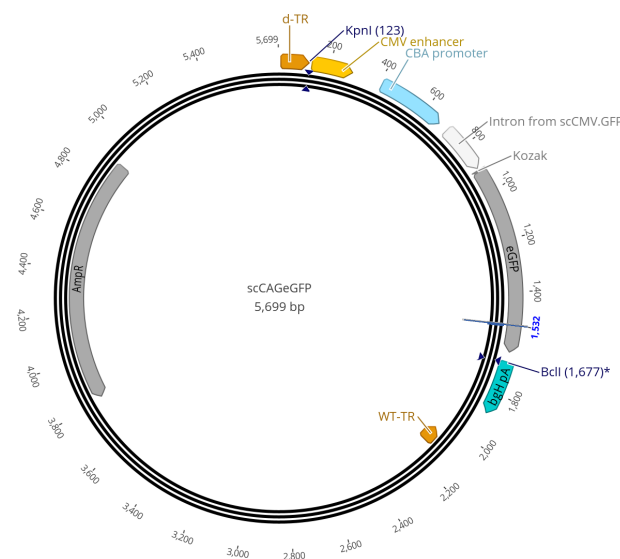
7m8(rep/cap):



**pHelper:**



scCAG-GFP-BC43:  
 scCAG-GFP-BC138:  
 scCAG-GFP-BC208:  
 scCAG-GFP-BC224:  
 scCAG-GFP-BC128:  
 scCAG-GFP-BC225:  
 scCAG-GFP-BC209:



### Packaging protocol and Titering:

Barcoded AAVs were purchased commercially from Packgene and diluted in-house. The titers were measured using qPCR AAV titer kit (ABM, catalog# G931), following the manufacturer's protocol.

AAV construct	Titer (Vg/mL)
7m8-CAG-BC43 GFP	1.00e+13
7m8-CAG-BC138 GFP	1.53e+12
7m8-CAG-BC208 GFP	2.44e+11
7m8-CAG-BC224 GFP	2.06e+10
7m8-CAG-BC128 GFP	1.21e+9
7m8-CAG-BC225 GFP	1.71e+8
7m8-CAG-BC209 GFP	4.22e+7
Final 7m8 library pool	2.99E+12

### Endotoxin levels:

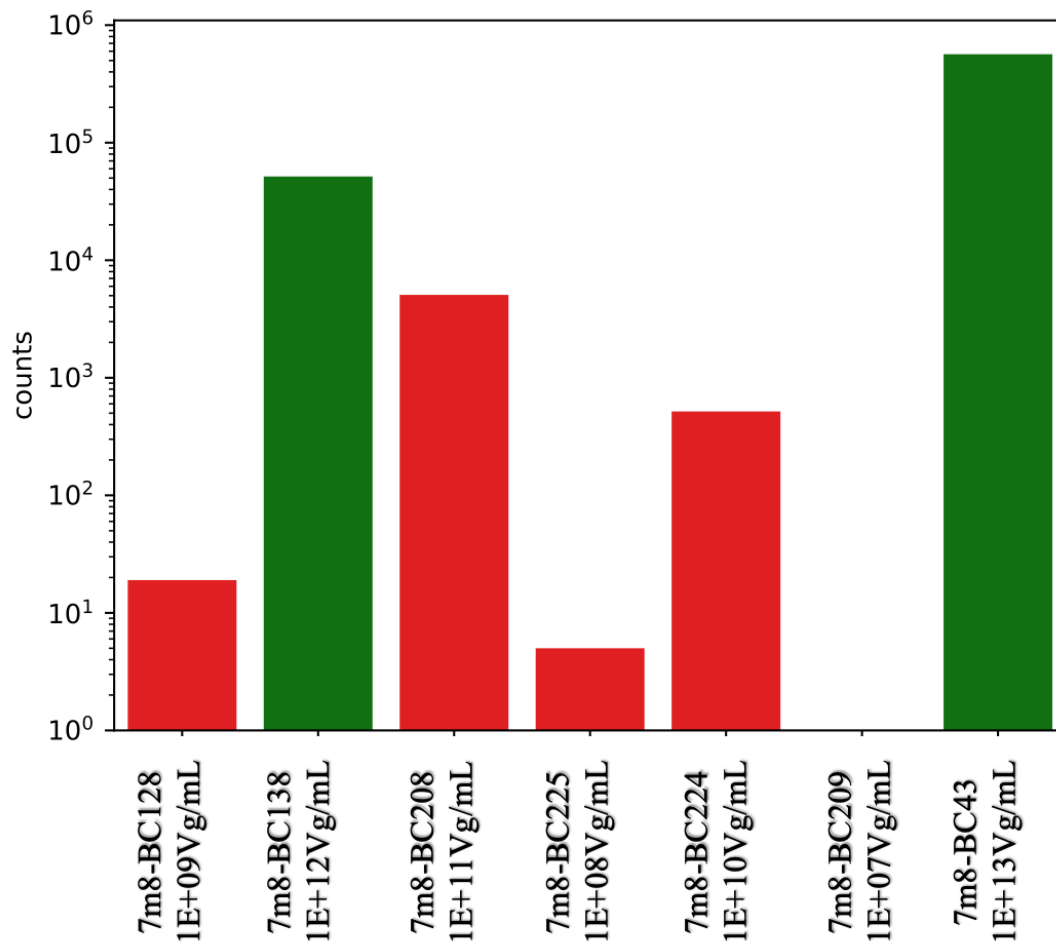
Bacterial endotoxin was detected by gel-clot method. The sample is diluted with water for BET to the maximum dilution factor. Take the sample dilution and mix it with an equal volume of LAL reagent (e.g., 0.1 ml aliquot). Continue incubating the reaction mixture ( at  $37 \pm 1$  °C for  $60 \pm 2$  min) without vibration. To test the integrity of the gel, remove each tube directly from the incubator and invert it through approximately 180° in one smooth motion. If a firm gel is formed that remains in place upon

inversion, record the result as positive. If no intact gel is formed, the result is negative.

AAV construct	Value (EU/mL)
7m8-CAG-BC43 GFP	<0.2
7m8-CAG-BC138 GFP	<0.2
7m8-CAG-BC208 GFP	<0.2
7m8-CAG-BC224 GFP	<0.2
7m8-CAG-BC128 GFP	<0.2
7m8-CAG-BC225 GFP	<0.2
7m8-CAG-BC209 GFP	<0.2
Final 7m8 library pool	<0.5

### Dilution curve analysis:

Final titer of the pooled dilution curve was 2.99E+12 vg/mL. Barcodes from the pooled library were amplified by PCR and then subjected to sequencing on an Illumina iSeq100. Quantification of barcodes revealed the following library balance:



QC provided by Packgene is copied below.

## Certificate of Analysis (COA)

<b>Name</b>	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 128]	<b>Lot#</b>	19341-2E1
<b>Order#</b>	USJW241008942-2	<b>Date</b>	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer: 4.38E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	<0.2EU/mL

### Conclusion:Qualified

\* Adjusted Titer: Titers adjusted to order requirements.

## Item 1: Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19341-2E1	3.18E+11	0.69	20	4.38E+12	4.38E+12

\* Reference AAV:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer: 5.5E+11(GC/mL)

Figure 1: Sample Location

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITR 8E14	ITR 8E14	ITR 8E14	19341-2 ITR 3.15E11	19341-2 ITR 3.21E11							
B	ITR 8E13	ITR 8E13	ITR 8E13									
C	ITR 8E12	ITR 8E12	ITR 8E12									
D	ITR 8E11	ITR 8E11	ITR 8E11									
E	ITR 8E10	ITR 8E10	ITR 8E10									
F	ITR 8E9	ITR 8E9	ITR 8E9									
G	Ref AAV ITR 8.09E11	Ref AAV ITR 7.83E11	Ref AAV ITR 8.04E11									
H	NC ITR 3.25E8	Dnase ITR 2.08E8										

Figure 2 Amplification curves

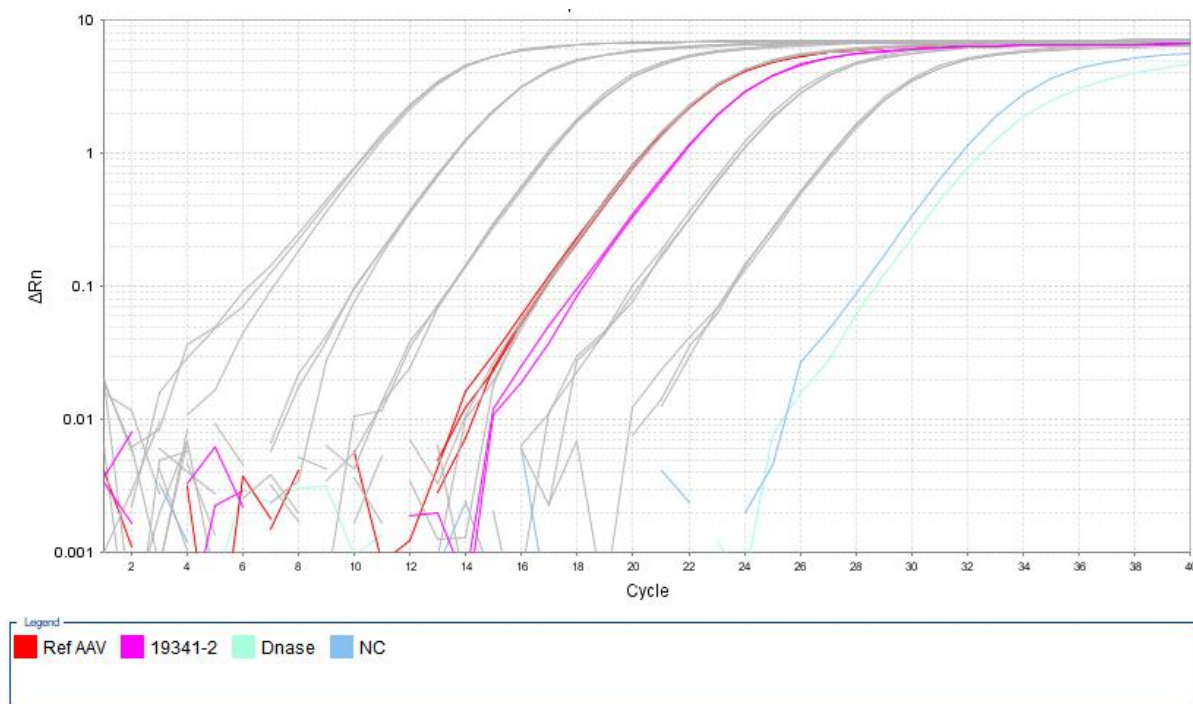
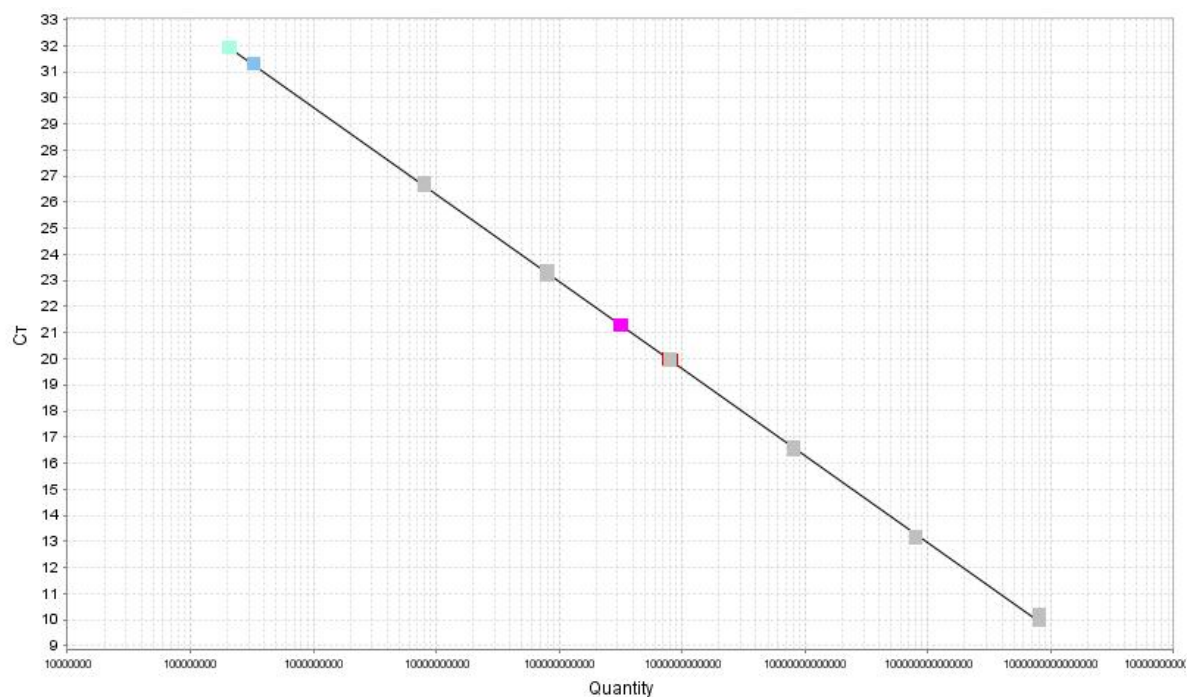


Figure 3 Standard Curve



Target: ITR Slope: -3.343 Y-Inter: 59.761  $R^2$ : 1 Eff%: 99.128

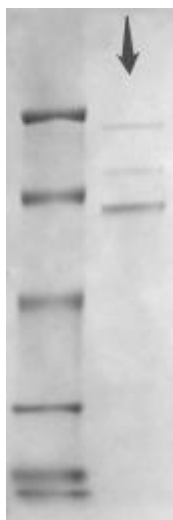
Legend  
 19341-2 Ref AAV not assigned Dnase NC

## Item 2: Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

## Item 3: Purity (SDS-PAGE)





**Maker:**97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

**Conclusion:** No other significant band

## Certificate of Analysis (COA)

<b>Name</b>	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 208]	<b>Lot#</b>	19343-2E1
<b>Order#</b>	USJW241008942-6	<b>Date</b>	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer: 5.67E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	<0.2EU/mL

**Conclusion:Qualified**

\* Adjusted Titer: Titers adjusted to order requirements.

## Item 1: Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19343-2E1	4.12E+11	0.69	20	5.67E+12	5.67E+12

\* Reference AAV:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer: 5.5E+11(GC/mL)

Figure 1: Sample Location

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITR 8E14	ITR 8E14	ITR 8E14									
B	ITR 8E13	ITR 8E13	ITR 8E13									
C	ITR 8E12	ITR 8E12	ITR 8E12	19343-2 ITR 4.03E11	19343-2 ITR 4.21E11							
D	ITR 8E11	ITR 8E11	ITR 8E11									
E	ITR 8E10	ITR 8E10	ITR 8E10									
F	ITR 8E9	ITR 8E9	ITR 8E9									
G	Ref AAV ITR 8.09E11	Ref AAV ITR 7.83E11	Ref AAV ITR 8.04E11									
H	NC ITR 3.25E8	Dnase ITR 2.08E8										

Figure 2 Amplification curves

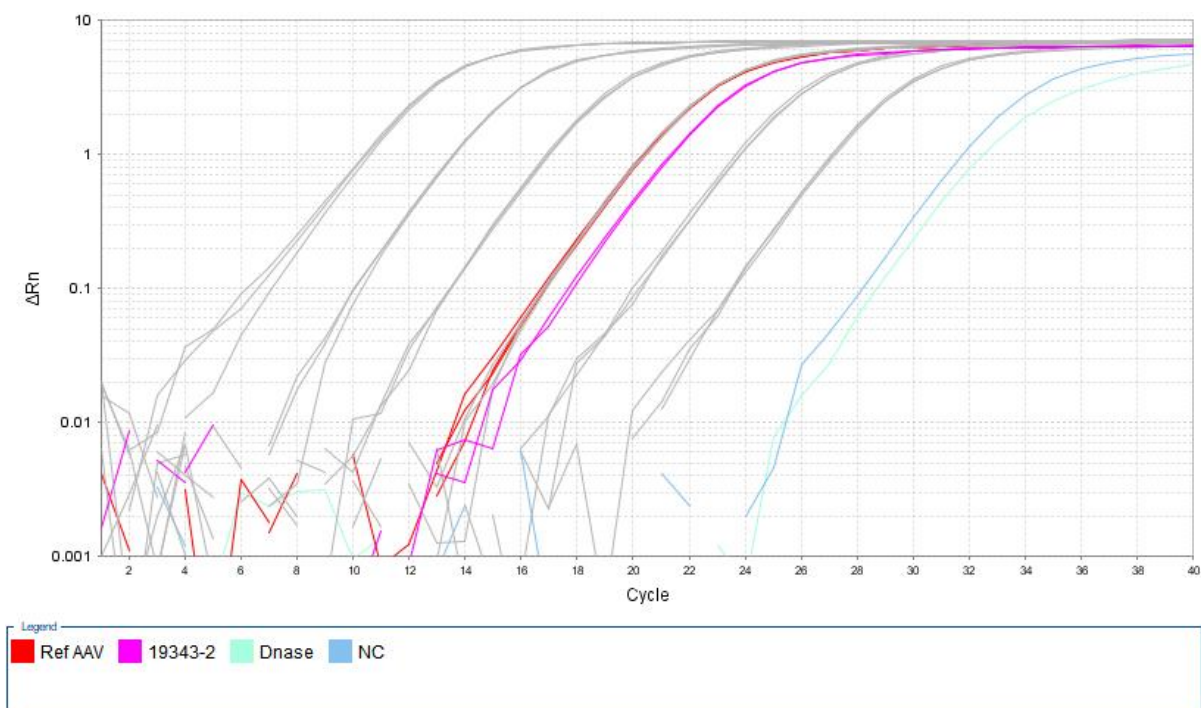
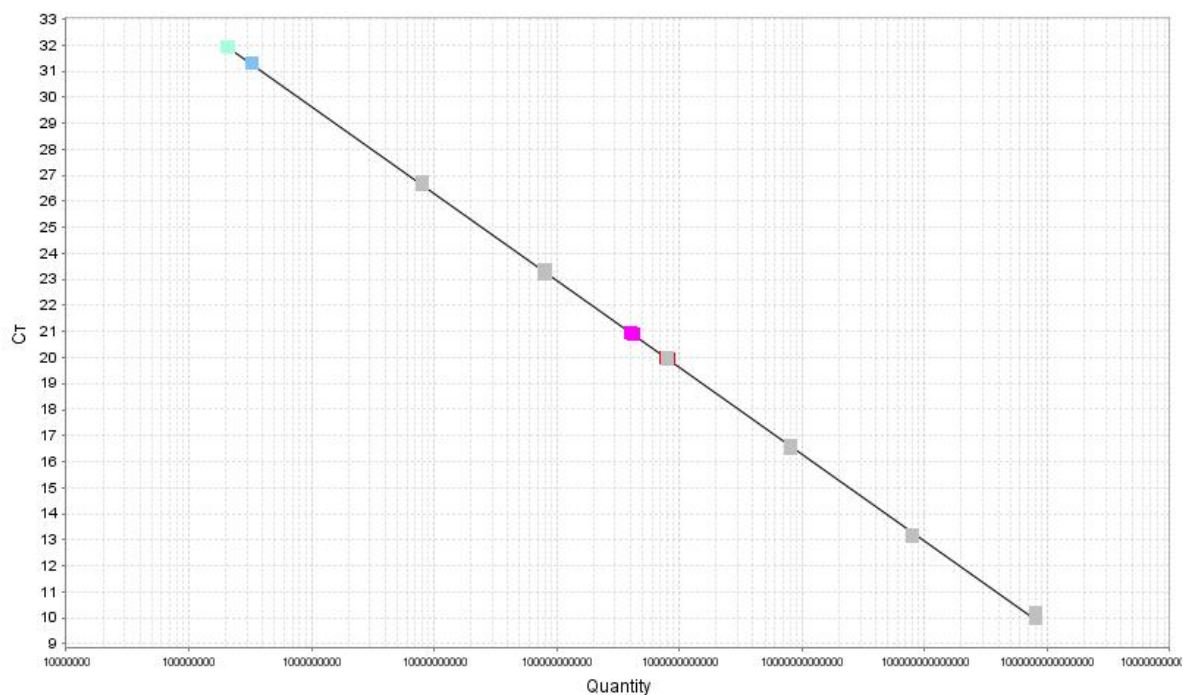


Figure 3 Standard Curve



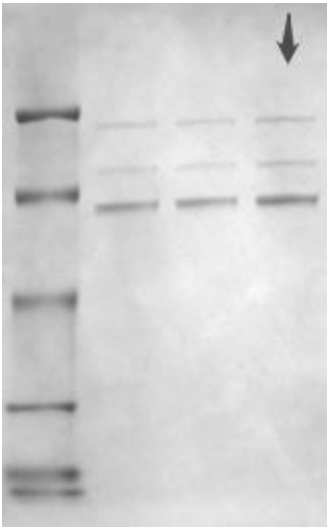
Target: ITR Slope: -3.343 Y-Inter: 59.761  $R^2$ : 1 Eff%: 99.128

Legend  
■ Ref AAV ■ 19343-2 ■ not assigned ■ Dnase ■ NC

## Item 2: Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

## Item 3: Purity (SDS-PAGE)



**Maker:**97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

**Conclusion:** No other significant band

## Certificate of Analysis (COA)

<b>Name</b>	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 209]	<b>Lot#</b>	19344-2E1
<b>Order#</b>	USJW241008942-8	<b>Date</b>	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer: 2.65E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	<0.2EU/mL

### Conclusion:Qualified

\* Adjusted Titer: Titers adjusted to order requirements.

## Item 1: Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19344-2E1	1.92E+11	0.69	20	2.65E+12	2.65E+12

\* Reference AAV:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer: 5.5E+11(GC/mL)

Figure 1: Sample Location

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITR 8E14	ITR 8E14	ITR 8E14									
B	ITR 8E13	ITR 8E13	ITR 8E13									
C	ITR 8E12	ITR 8E12	ITR 8E12									
D	ITR 8E11	ITR 8E11	ITR 8E11	19344-2 ITR 1.87E11	19344-2 ITR 1.97E11							
E	ITR 8E10	ITR 8E10	ITR 8E10									
F	ITR 8E9	ITR 8E9	ITR 8E9									
G	Ref AAV ITR 8.09E11	Ref AAV ITR 7.83E11	Ref AAV ITR 8.04E11									
H	NC ITR 3.25E8	Dnase ITR 2.08E8										



Figure 2 Amplification curves

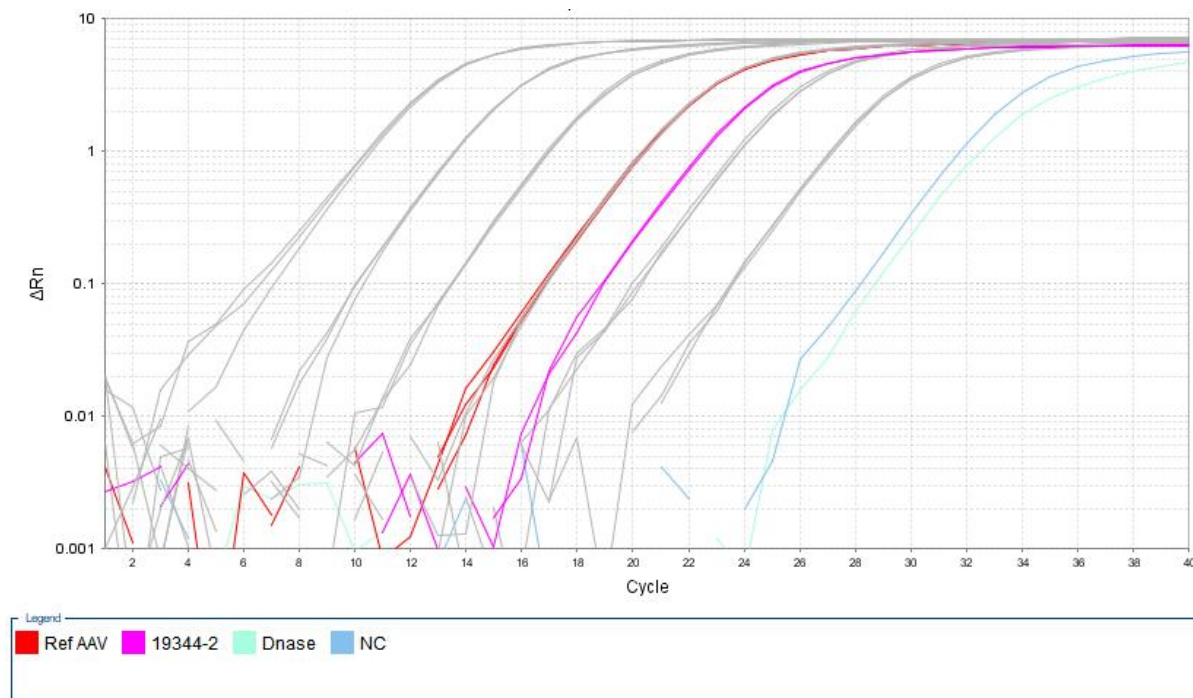
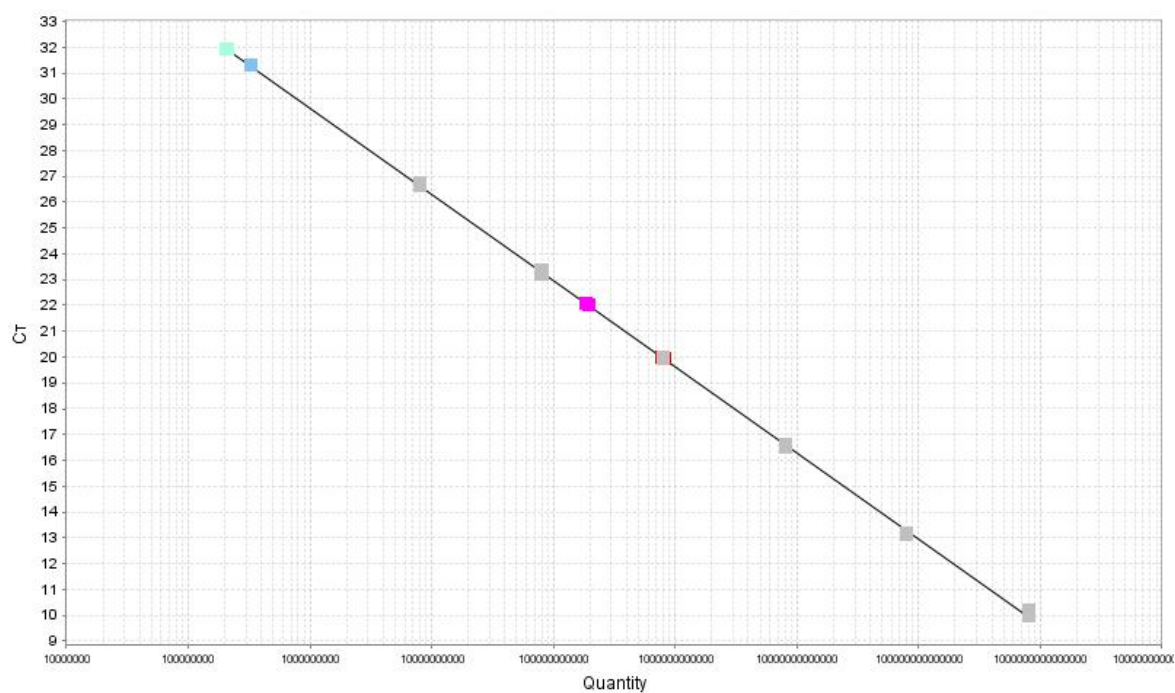


Figure 3 Standard Curve



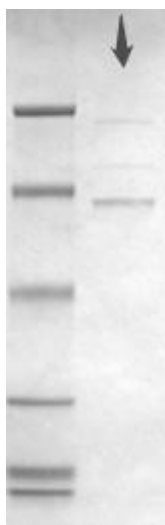
Target: ITR Slope: -3.343 Y-Inter: 59.761  $R^2$ : 1 Eff%: 99.128

Legend  
■ 19344-2 ■ Ref AAV ■ not assigned ■ Dnase ■ NC

## Item 2: Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

## Item 3: Purity (SDS-PAGE)



**Maker:**97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

**Conclusion:** No other significant band

## Certificate of Analysis (COA)

<b>Name</b>	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 224]	<b>Lot#</b>	19345-2E1
<b>Order#</b>	USJW241008942-10	<b>Date</b>	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer: 5.61E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	<0.2EU/mL

**Conclusion:Qualified**

\* Adjusted Titer: Titers adjusted to order requirements.

## Item 1: Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19345-2E1	4.07E+11	0.69	20	5.61E+12	5.61E+12

\* Reference AAV:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer: 5.5E+11(GC/mL)

Figure 1: Sample Location

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITR 8E14	ITR 8E14	ITR 8E14									
B	ITR 8E13	ITR 8E13	ITR 8E13									
C	ITR 8E12	ITR 8E12	ITR 8E12									
D	ITR 8E11	ITR 8E11	ITR 8E11									
E	ITR 8E10	ITR 8E10	ITR 8E10	19345-2 ITR 4.07E11	19345-2 ITR 4.08E11							
F	ITR 8E9	ITR 8E9	ITR 8E9									
G	Ref AAV ITR 8.09E11	Ref AAV ITR 7.83E11	Ref AAV ITR 8.04E11									
H	NC ITR 3.25E8	Dnase ITR 2.08E8										

Figure 2 Amplification curves

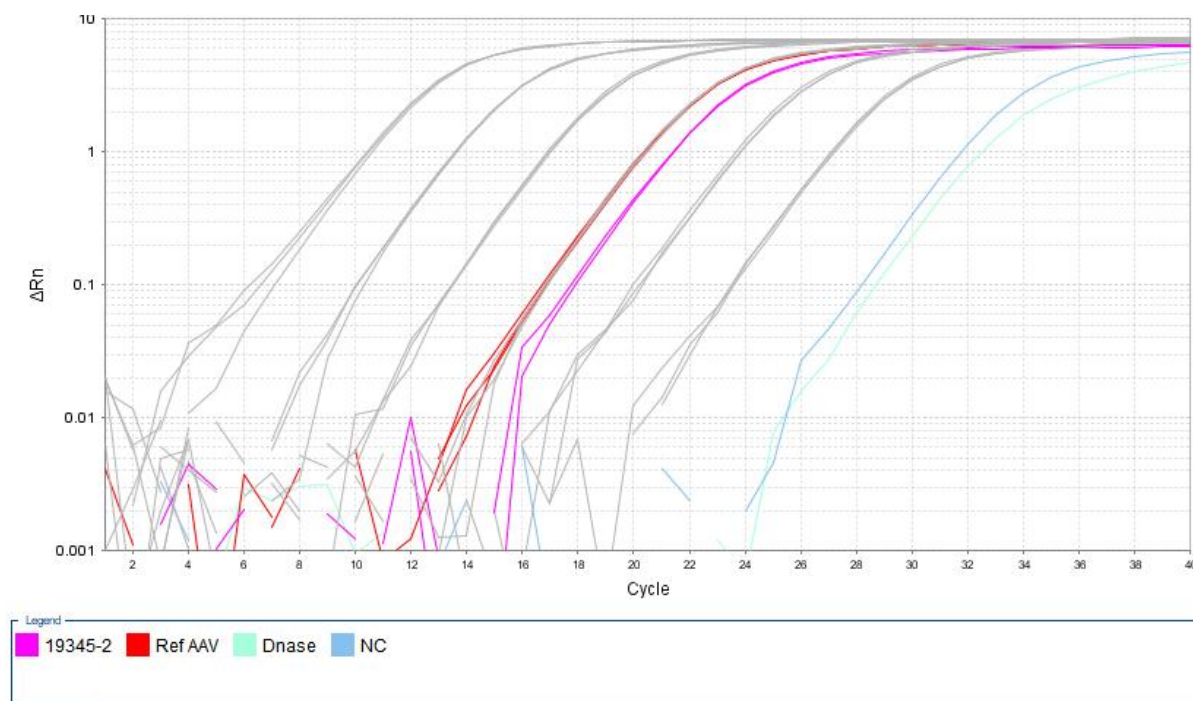
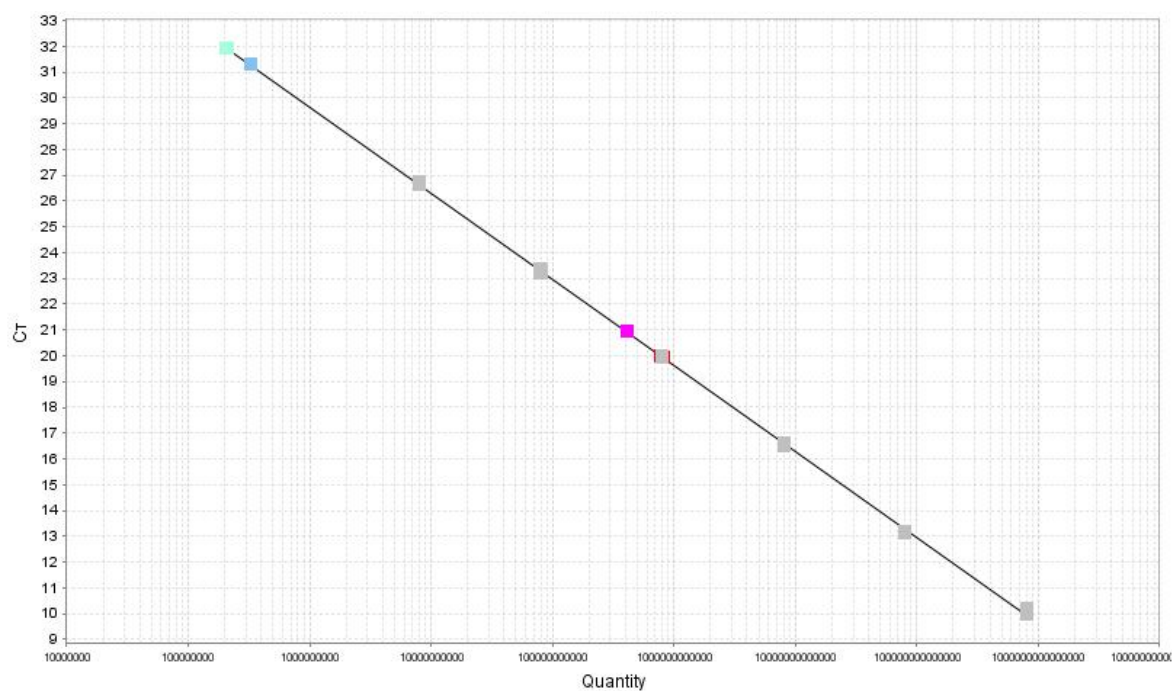


Figure 3 Standard Curve



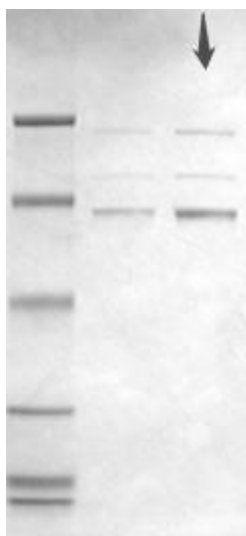
Target: ITR Slope: -3.343 Y-Inter: 59.761  $R^2$ : 1 Eff%: 99.128

Legend  
■ Ref AAV ■ not assigned ■ Dnase ■ 19345-2 ■ NC

## Item 2: Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

## Item 3: Purity (SDS-PAGE)



**Maker:**97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

**Conclusion:** No other significant band



## Certificate of Analysis (COA)

<b>Name</b>	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 225]	<b>Lot#</b>	19346-2E1
<b>Order#</b>	USJW241008942-12	<b>Date</b>	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer: 4.36E+12GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	<0.2EU/mL

### Conclusion:Qualified

\* Adjusted Titer: Titers adjusted to order requirements.

## Item 1: Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19346-2E1	3.17E+11	0.69	20	4.36E+12	4.36E+12

\* Reference AAV:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer: 5.5E+11(GC/mL)

Figure 1: Sample Location

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITR 8E14	ITR 8E14	ITR 8E14									
B	ITR 8E13	ITR 8E13	ITR 8E13									
C	ITR 8E12	ITR 8E12	ITR 8E12									
D	ITR 8E11	ITR 8E11	ITR 8E11									
E	ITR 8E10	ITR 8E10	ITR 8E10									
F	ITR 8E9	ITR 8E9	ITR 8E9	19346-2 ITR 3.16E11	19346-2 ITR 3.18E11							
G	Ref AAV ITR 8.09E11	Ref AAV ITR 7.83E11	Ref AAV ITR 8.04E11									
H	NC ITR 3.25E8	Dnase ITR 2.08E8										

Figure 2 Amplification curves

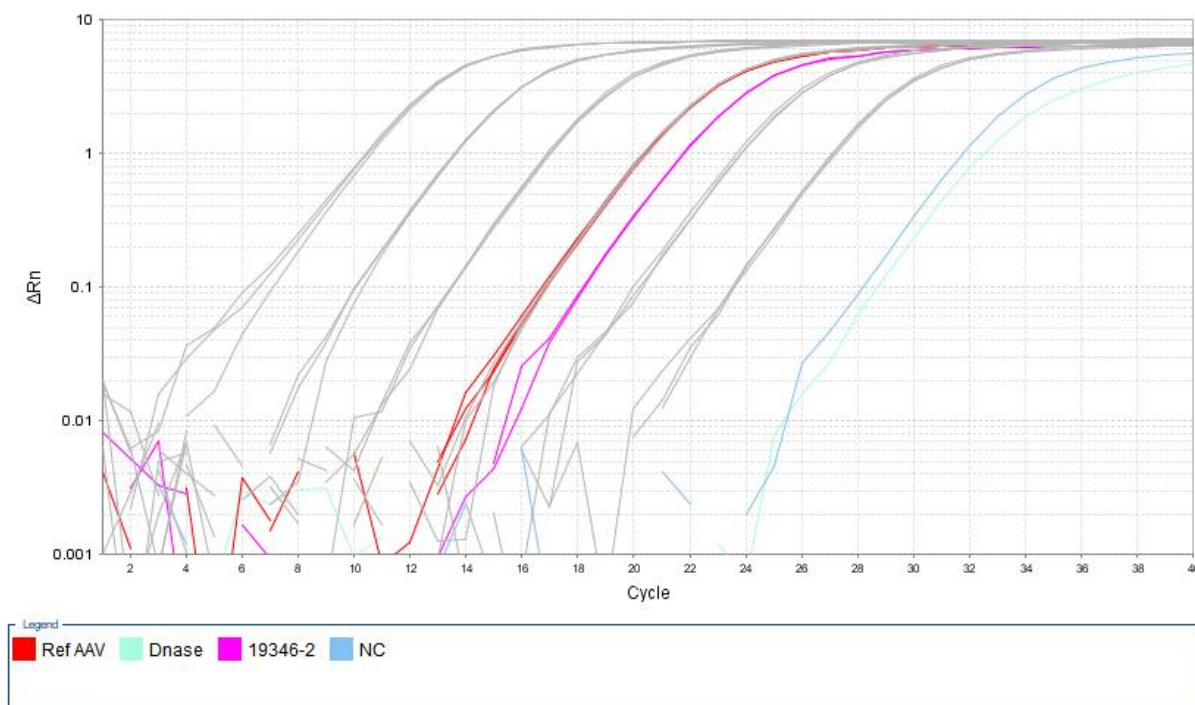
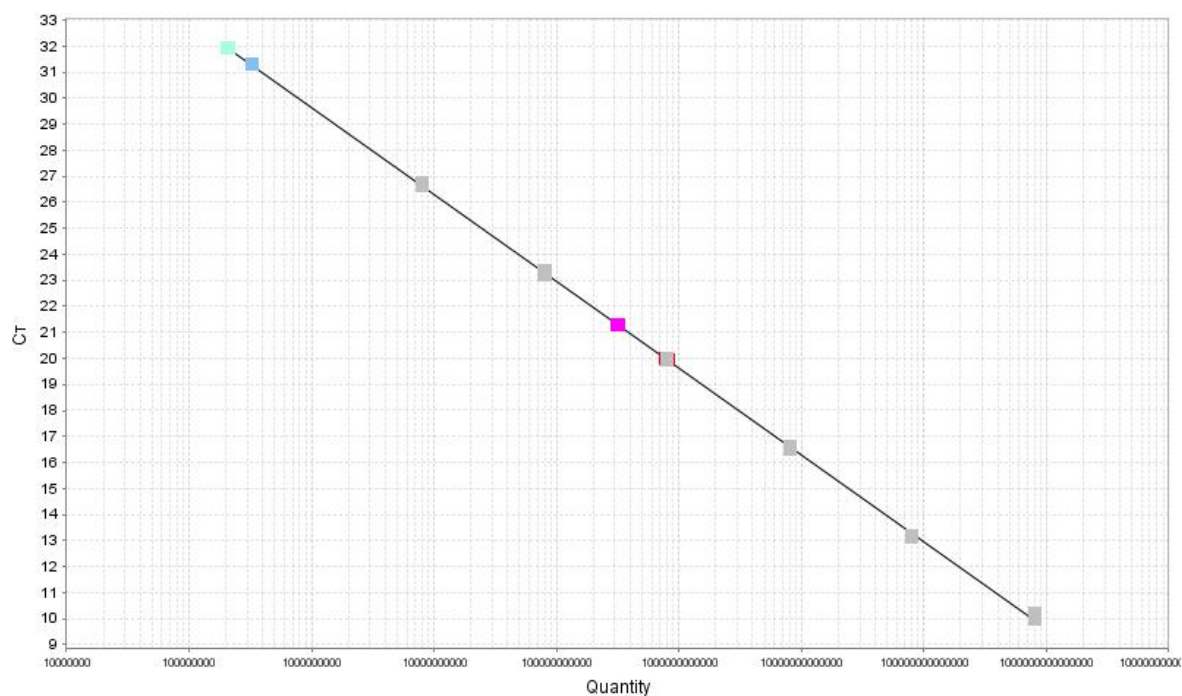


Figure 3 Standard Curve



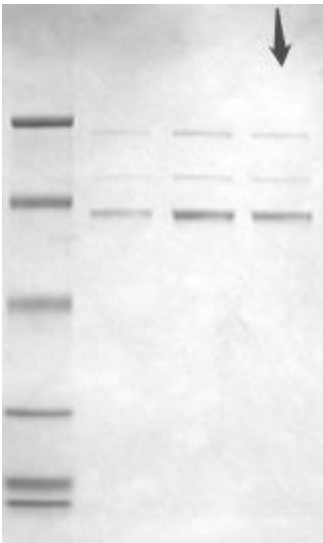
Target: ITR Slope: -3.343 Y-Inter: 59.761  $R^2$ : 1 Eff%: 99.128

Legend  
■ Ref AAV ■ 19346-2 ■ not assigned ■ Dnase ■ NC

## Item 2: Endotoxin

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

## Item 3: Purity (SDS-PAGE)



**Maker:**97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

**Conclusion:** No other significant band

## Certificate of Analysis (COA)

<b>Name</b>	7m8 (Engineered AAV2, contains loop insertion)[CAG-GFP.Barcode 43]	<b>Lot#</b>	19368-2T
<b>Order#</b>	USJW241008942-84	<b>Date</b>	2024-11-15

Items	Analytical Method	QC results
[Genome Titer]	qPCR	Adjusted Titer: 1E+13GC/mL
[Purity]	SDS-PAGE	No other significant band
[Endotoxin]	LAL	<0.2EU/mL

### Conclusion:Qualified

\* Adjusted Titer: Titers adjusted to order requirements.

## Item 1: Genome Titer

Lot#	Quantity Mean GC/ml	Calibration Factor	Dilution factor*	Detected Titer(GC/mL)	Adjusted Titer (GC/mL)
19368-2T	1.40E+12	0.52	20	1.44E+13	1E+13

\* Reference AAV:Packgene:AAV2[ssAAV.CAG.EGFP.WPRE.SV40pA],Labeled titer: 5.5E+11(GC/mL)

Figure 1: Sample Location

	1	2	3	4	5	6	7	8	9	10	11	12
A	ITR 8E14	ITR 8E14	ITR 8E14					19368-2 ITR 1.41E12	19368-2 ITR 1.38E12			
B	ITR 8E13	ITR 8E13	ITR 8E13									
C	ITR 8E12	ITR 8E12	ITR 8E12									
D	ITR 8E11	ITR 8E11	ITR 8E11									
E	ITR 8E10	ITR 8E10	ITR 8E10									
F	ITR 8E9	ITR 8E9	ITR 8E9									
G	Ref AAV ITR 1.11E12	Ref AAV ITR 1.03E12	Ref AAV ITR 1.06E12									
H	NC ITR 3.15E8	Dnase ITR 8.55E7										



Figure 2 Amplification curves

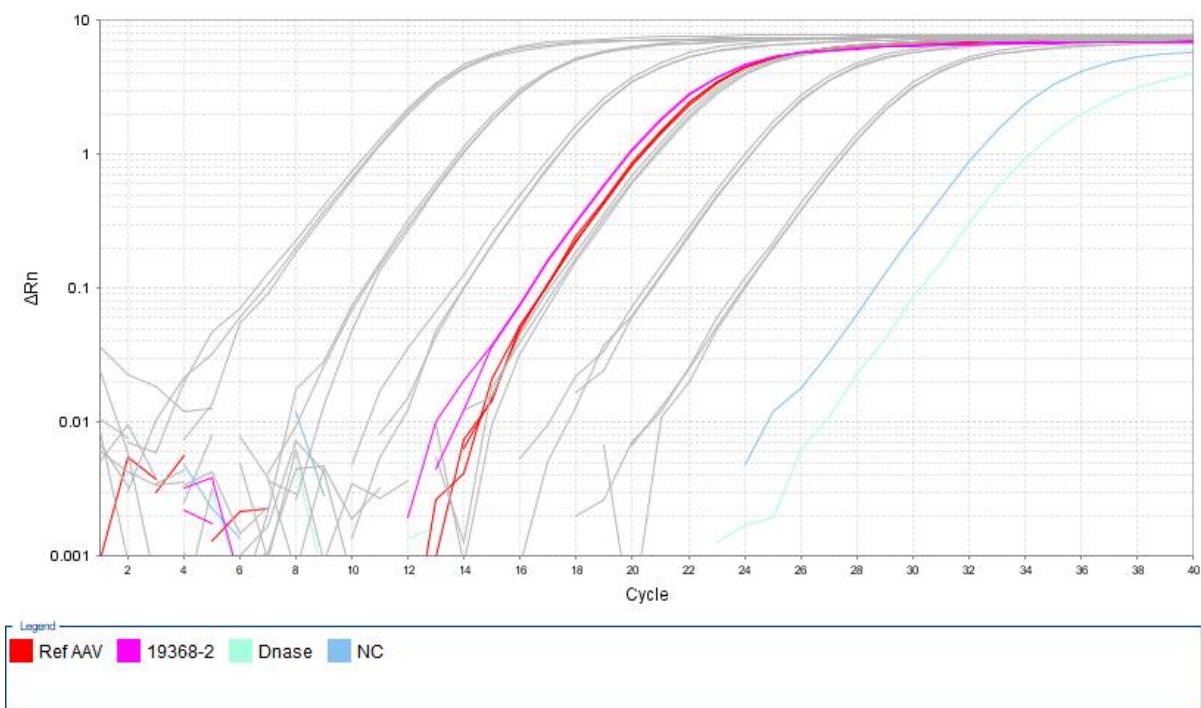
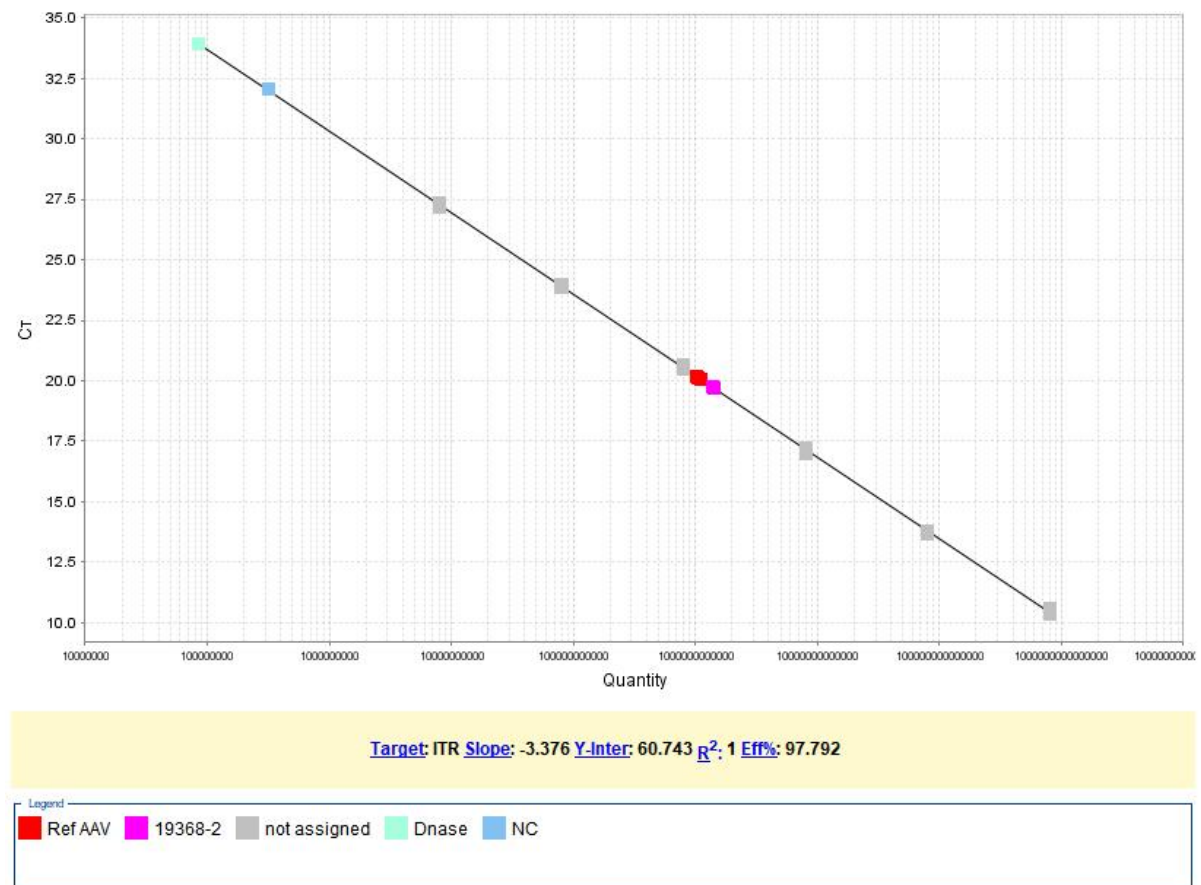


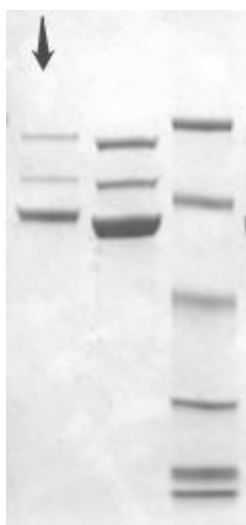
Figure 3 Standard Curve





**Item 2: Endotoxin**

Name	Sample positive control	Positive control	Neg control	Sample
Result	+	+	—	—

**Item 3: Purity (SDS-PAGE)**

**Maker:**97.2KD,66.4KD,44.3KD,29KD,20.1KD,14.3KD

**Conclusion:** No other significant band