

AAV Batch #: AAV2-04172024-scCAG-mgL

Serotype: AAV2

GOI: pAAV-scCAG-mgL

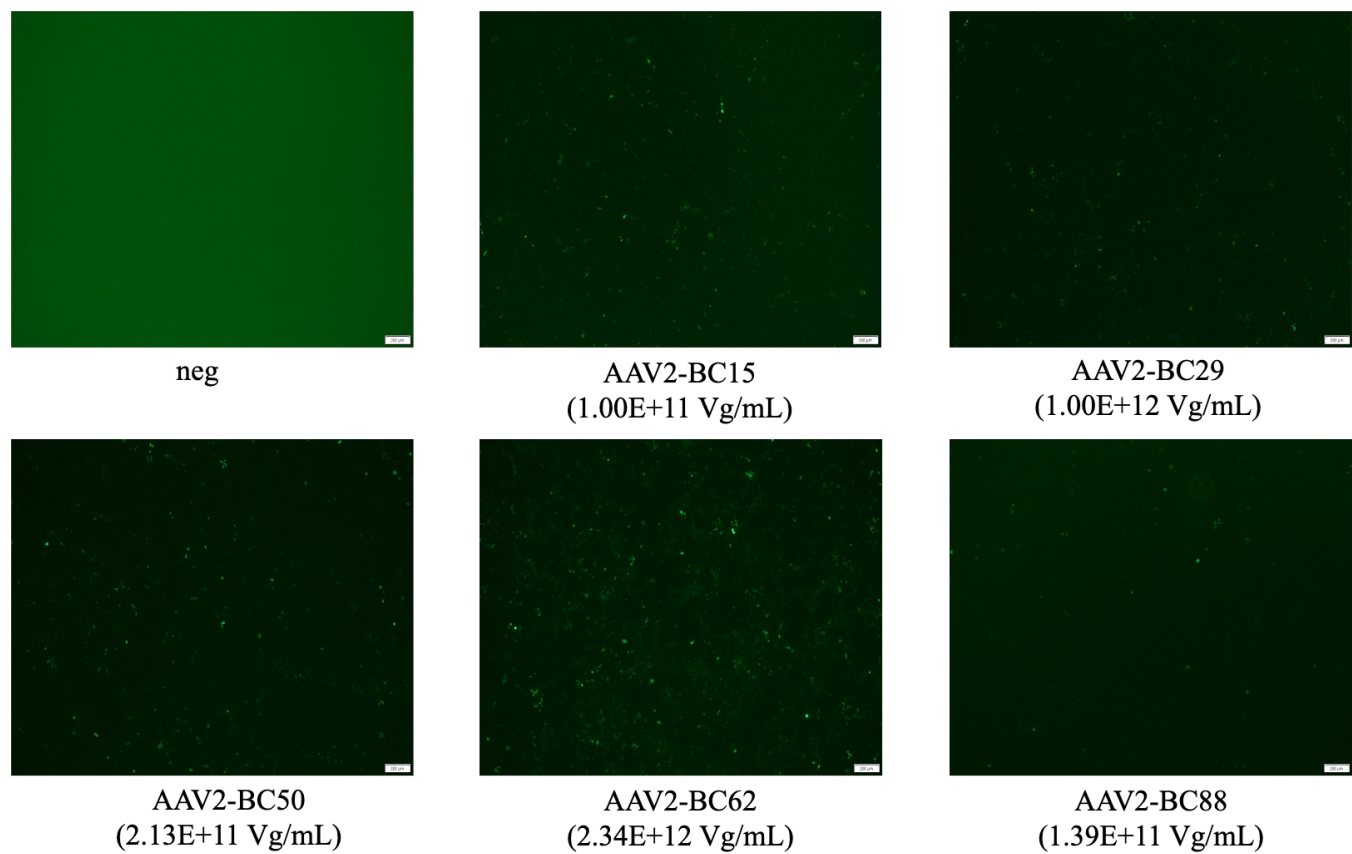
Plasmid map:

Packaging protocol:

Barcoded AAVs were packaged using a triple transfection method in adherent cells.

In vitro infectivity test:

1 uL of packaged virus was used to infect 1 well of a 6-well plate containing HEK293 cells at 70% confluency.



Titering:

The titers were measure using qPCR AAV titer kit (catalog# G931), following the manufacturer’s protocol.

AAV construct	Titer (Vg/mL)
AAV2-CAG-BC15 1 mgL	1.00E+11
AAV2-CAG-BC29 2 mgL	1.00E+12
AAV2-CAG-BC50 1 mgL	2.13E+11
AAV2-CAG-BC62 mgL	2.34E+12
AAV2-CAG-BC88_1 mgL	1.39E+11

Mass photometry:

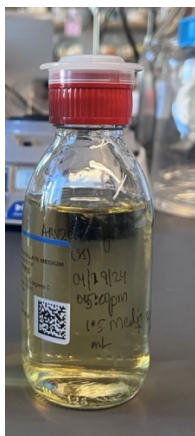
	Construct 1	Construct 2	Construct 3	Construct 4	Construct 5
% full	94.2	96	92.9	92.2	95.1
% empty	5.8	4	6.8	7.8	4.4
% ambiguous	0.0	0.2	0.3	0.1	0.5

Fungal and bacterial contamination:

2 μ L of purified AAV was tested for bacterial and fungal contamination using qPCR-based Femto Bacterial DNA Quantification Kit (catalog # E2006) and Femto Fungal DNA Quantification Kit (catalog # E20067), following manufacturer's instructions. A $C_t > 30$ was considered no contamination.

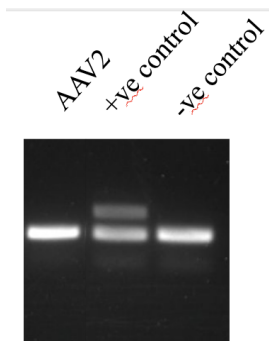
Test	AAV2 library mix
Fungus (C_t)	39.335
Bacteria (C_t)	34.197

1 μ L of purified AAV was also tested in 100 mL of FTM media, culturing at 37 $^{\circ}$ C for 14 days. Media was clear and free of contamination.



Mycoplasma:

The final library pool was investigated for mycoplasma contamination, using Myco-Sniff-Valid Mycoplasma PCR Detection kit (Catalog# 093050301), following the manufacturer's protocol.



Endotoxin:

The final library pool was investigated for endotoxin contamination. The samples were submitted for investigation to Charles River Laboratories.

SAMPLE RESULTS:

Serial Number/Bay: MCS 19211061/1			Start/End Temp (°C): 37.0/37.0					
Sample Name: AAV2 Library								
Sample ID: N/A			Sample Lot: N/A		Dil./Conc.: 1:500 mL/mL			
Dilution: N/A								
Sample Comments: N/A								
Cartridge Lot Number: 4516155			Cartridge Range: 0.5-0.005 EU/mL					
Calibration Code: 418156542390			Archived Spike Concentration: 0.043 EU/mL					
Range: 181-965 seconds			Y-Intercept: +2.148	Slope: -0.363				
Endotoxin Value: 78.3 EU/mL			Sample CV Limit: <25%	Status: VALID				
Endotoxin Limit: No Limit	Status: N/A		Spike CV Limit: <25%	Status: VALID				
Alert Limit: N/A			Spike Recovery Range: 50-200%	Status: VALID				
Cartridge Type: LAL Cartridge			Performed With: N/A					
SAMPLE DATA								
Channel	Reaction Time	CV%	Sample Value	Channel	Reaction Time	CV%	Spike Value	Spike Recovery %
1	266	5.1%	78.3 EU/mL	2	246	2.3%	0.0490 EU/mL	114%
3	286			4	254			

Dilution curve analysis:

Final titer of the pooled dilution curve was 2.48E+11 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:

X-axis label		dilution (Vg/mL)	counts_1	counts_2	counts_3	Avg counts	Std dev
1	BC62	1.00E+11	576735	437692	479533	497987	71335
2	BC50	1.00E+10	43890	53560	59722	48725	6838
3	BC29	1.00E+09	6218	5153	5316	5562	574
4	BC15	1.00E+08	1798	1437	1489	1575	195
5	BC88	1.00E+07	120	154	157	144	21

