Evaluating AAV Neutralizing Antibodies With a High-Sensitivity LacZ Reporter Assay

Booth 1821

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Introduction

Adeno-associated viruses (AAVs) are a cornerstone of therapeutic gene delivery. A significant challenge to their success is the presence of pre-existing AAV neutralizing antibodies (nAbs) in patients, which can diminish the effectiveness and safety of AAV-based treatments. Furthermore, the immune response induced by the administered AAV vector can lead to the development of new nAbs, complicating long-term treatment strategies. Consequently, robust methods for quantifying nAb levels in patients both before and after AAV gene therapy are crucial for clinical translation.

This study introduces a novel LacZ reporter system, uniquely tailored for multiple AAV serotypes, to facilitate sensitive and efficient cell-based detection of AAV neutralizing antibodies. This innovative assay provides a powerful and reliable platform for evaluating the immunogenicity of AAV gene therapies, offering a more streamlined approach to nAb detection than traditional methods.

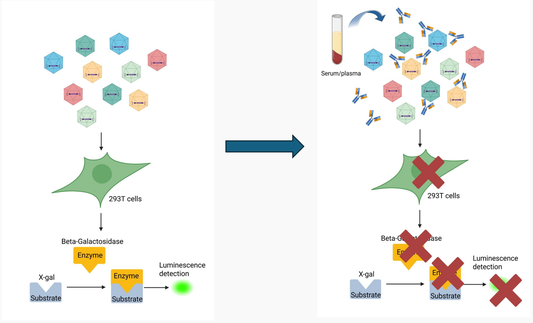
Plasmid Carrying LacZ as GOI

ssAAV.CMV.LacZ.WPREs.SV40pA GOI plasmid

AAV2, AAV5, AAV8, AAV9, etc

We have designed a gene of interest (GOI) plasmid, named ssAAV.CMV.LacZ.WPREs.SV40pA, that is engineered to express the LacZ reporter gene. This plasmid can then be used to package the LacZ gene into AAV viral particles with different capsid serotypes.

Assay Workflow: Neutralizing Antibodies Blocking AAV infection and Reduce β-Galactosidase Expression



rAAV serotypes 2, 5, 8, and 9, each encapsidating the ssAAV.CMV.LacZ.WPREs.SV40pA expression cassette, were used to transduce 293T cells pre-seeded in 96-well plates. After a 48-hour incubation period to allow for LacZ transgene expression, cells were harvested, and β-galactosidase activity was quantified using the Galacto-Star™ β-Galactosidase Reporter Gene Assay.

The assay quantifies the extent of residual AAV transduction following pre-incubation with test serum. The resulting luminescence signal, generated by β-galactosidase activity in transduced cells, exhibits an inverse relationship with the concentration of nAbs present in the serum sample. Higher luminescence indicates lower nAb levels, and vice versa.

Reduction in β-Galactosidase Activity Reflects Neutralizing Antibody Inhibition of rAAV Infectivity

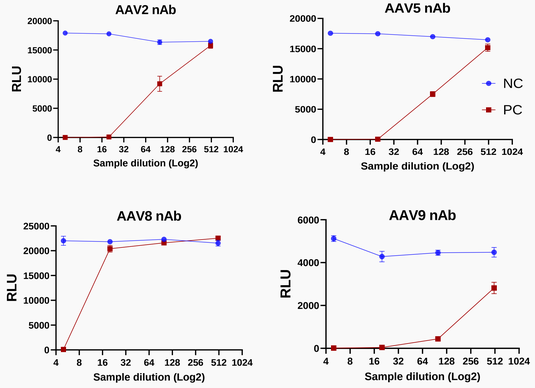
Animals were immunized with distinct rAAV capsid serotypes to generate anti-rAAV sera, which served as positive controls (PC). Sera from non-immunized (naïve) animals were used as negative controls (NC). Both PC and NC sera were tested at various dilution levels. PC sera raised against AAV2, AAV5, AAV8, and AAV9 exhibited dose-dependent decreases in activity with increasing dilution. In contrast, NC sera showed no significant inhibitory effect across the tested dilutions. Triplicate relative light unit (RLU) measurements for all four serotype-specific PC and corresponding NC samples at four different dilution levels demonstrated acceptable precision.

AAV2 nAb	Negative Control			Positive Control		
5	17517	18077	18151	15	14	15
20	17650	17955	17727	97	82	85
100	16578	16556	15884	8841	10651	8135
500	16741	16141	16597	16172	15933	15237

AAV5 nAb	Negative Control			Positive Control		
5	17797	17648	17238	13	15	12
20	17357	17603	17452	66	69	57
100	17078	16946	16928	7908	7547	7052
500	16734	16097	16588	15370	14494	15646

AAV8 nAb	Negative Control			Positive Control		
5	21865	21171	22982	90	114	104
20	22071	21485	21931	21129	19786	20294
100	22317	22158	22457	21565	21558	21661
500	22093	21557	20931	22563	22016	23000

AAV9 nAb	Negative Control			Positive Control		
5	4991	5115	5262	17	10	12
20	4548	4061	4231	39	34	53
100	4514	4325	4548	473	475	383
500	4237	4510	4693	3041	2888	2520



Assay Quantifies Neutralizing Antibody Inhibition of rAAV Infectivity with High Specificity and Precision

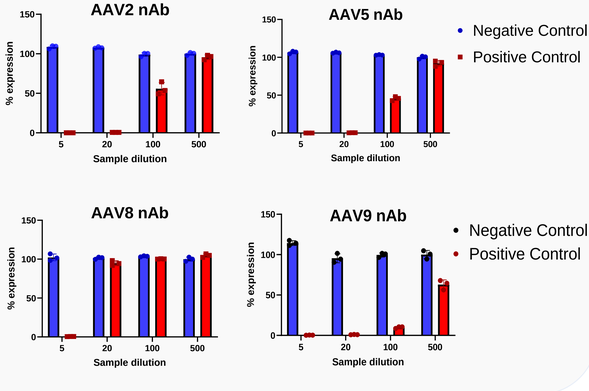
AAV2 nAb	Negative Control			Positive Control		
5	106.2%	109.6%	110.1%	0.1%	0.1%	0.1%
20	107.0%	108.9%	107.5%	0.6%	0.5%	0.5%
100	100.5%	100.4%	96.3%	53.6%	64.6%	49.3%
500	101.5%	97.9%	100.6%	98.1%	96.6%	92.4%

AAV5 nAb	Negative Control			Positive Control		
5	108.0%	107.1%	104.6%	0.1%	0.1%	0.1%
20	105.4%	106.9%	105.9%	0.4%	0.4%	0.3%
100	103.7%	102.9%	102.8%	48.0%	45.8%	42.8%
500	101.6%	97.7%	100.7%	93.3%	88.0%	95.0%

AAV8 nAb	Negative Control			Positive Control		
5	101.6%	98.3%	106.8%	0.4%	0.5%	0.5%
20	102.5%	99.8%	101.9%	98.2%	91.9%	94.3%
100	103.7%	102.9%	104.3%	100.2%	100.1%	100.6%
500	102.6%	100.1%	97.2%	104.8%	102.3%	106.8%

AAV9 nAb	Negative Control			Positive Control		
5	111.4%	114.2%	117.5%	0.4%	0.2%	0.3%
20	101.5%	90.6%	94.4%	0.9%	0.8%	1.2%
100	100.8%	96.5%	101.5%	10.6%	10.6%	8.5%
500	94.6%	100.7%	104.8%	67.9%	64.5%	56.3%

The luminescence signal of each sample was compared to the mean luminescence of a 500-fold diluted negative control for each AAV serotype. The percentage expression value quantifies the reduction in LacZ expression. For all four anti-rAAV positive control sera, 5-fold diluted samples reduced rAAV infectivity by over 99%. The IC50 dilution values for anti-AAV2, anti-AAV5, and anti-AAV9 PC sera were 91, 101, and 390, respectively. In contrast, anti-AAV8 PC sera showed no clear inhibitory effect at dilutions above 5-fold, indicating a lower nAb titer.



Summary

The PackGene LacZ Reporter Assay effectively quantifies the inhibitory effect of AAV nAbs. Demonstrating acceptable precision and antibody titer-dependent responses, this assay provides a valuable platform for evaluating the presence of AAV nAbs in biological samples.

Reference

Meliani A, et al., Determination of anti-adeno
associated virus vector neutralizing antibody titer with an in vitro reporter system. Hum Gene Ther Methods. 2015

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