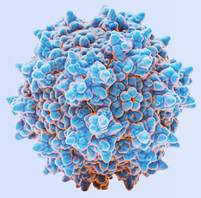
Evaluating AAV Neutralizing Antibodies With a High-Sensitivity LacZ Reporter Assay



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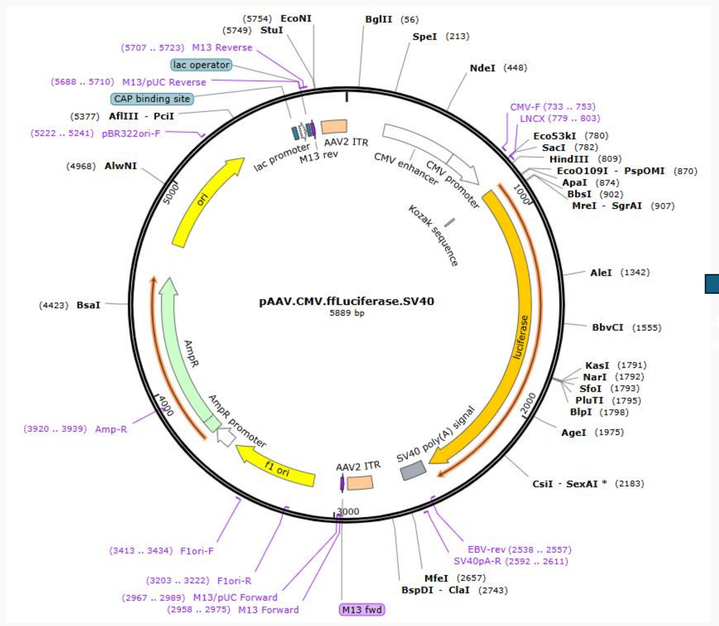
Introduction

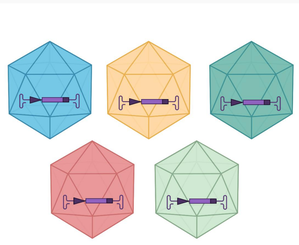
Adeno-associated viruses (AAVs) are a cornerstone of therapeutic gene delivery. A significant challenge to their success is the presence of pre-existing AAV neutralizing antibodies (nAbs) in patients, which can diminish the effectiveness and safety of AAV-based treatments. Furthermore, the immune response 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

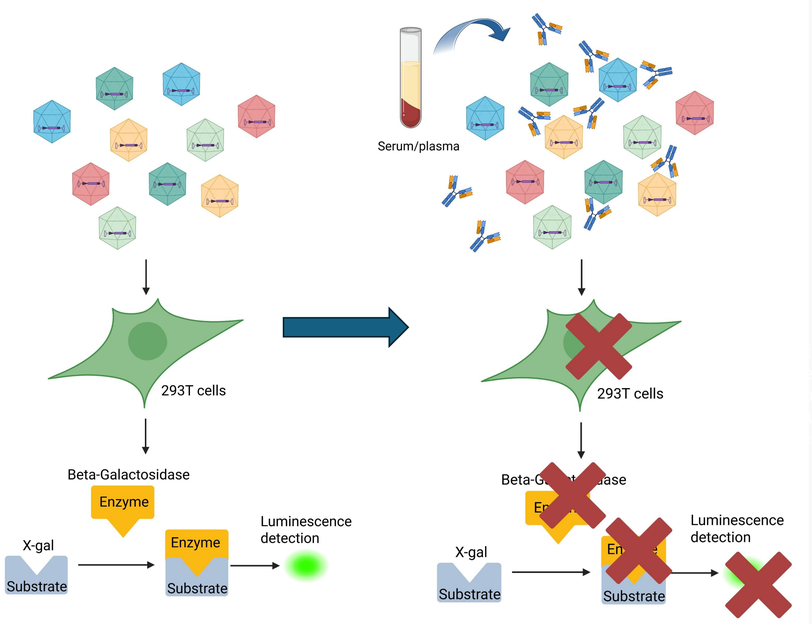




AAV2, AAV5, AAV8, AAV9, etc

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.

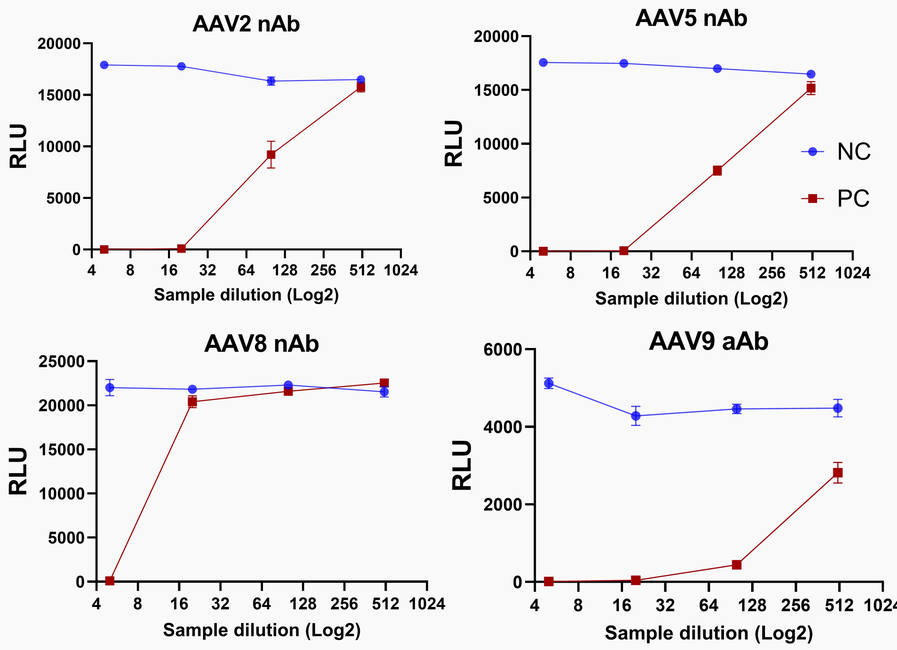
Assay Workflow: Neutralizing Antibodies Blocking AAV infection and Reduce β-Galactosidase Expression



The *lacZ* reporter 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.

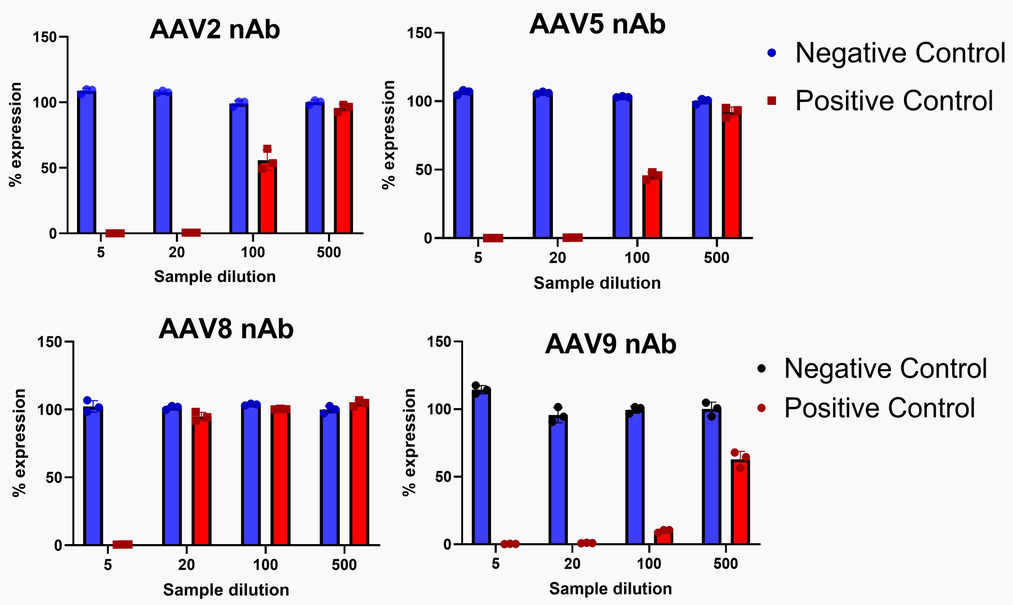
rAAV serotypes 2, 5, 8, and 9, each encapsidating the ssAAV.CMV.LacZ.WPREs.SV40pA expression cassette, were used to transduce 293T cells pre-seeded in 96-well plates. After a 48-hour incubation period to allow for *LacZ* transgene expression, cells were harvested, and β-galactosidase activity was quantified using the Galacto-Star™ β-Galactosidase Reporter Gene Assay. The presence of nAbs specific to an AAV serotype leads to the abrogation of viral transduction, thus inhibiting *LacZ* expression.

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.

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



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 Apr;26(2):45-53.

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