

Product datasheet

AAV8 Titration ELISA

Short overview

Cat. No.	PRAAV8
Quantity	96 tests

Product description

Application	ELISA
Product description	Unique microtiter plate enzyme immunoassay for the quantitation of virions and or assembled empty capsids of AAV8. The capture-antibody detects a conformational epitope not present on unassembled capsid proteins.
Kit control/Standard	AAV8 empty capsids; lyophilized
Form supplied	Reagent kit, 12 x 8-well strips
Storage	2-8°C
Intended use	Research use only
Reactivity	AAV8

Background

The ELISA principle:

The assay is based on the sandwich ELISA technique where a monoclonal antibody (mab) specific for a conformational epitope on assembled AAV capsids is coated onto the plate and is used to capture AAV particles from the specimen.

The detection of captured AAV particles is a two-step process.

- A biotin-conjugated mab is bound to the captured AAV particles.
- A streptavidin peroxidase conjugate reacts with the biotin molecules. The addition of the substrate results in a color reaction which is proportional to the amount of specifically bound viral particles.

Comparison of AAV Quantification Methods:

Each of the commonly used quantification methods has its pros and cons:

- qPCR is widely used, but suffers from several issues such as sample preparation, primer design, or PCR efficiency that can lead to high inter-laboratory variation of results.

- Digital droplet PCR methods overcome some of the limitations of qPCR. However, variations between labs can still occur due to different sample processing protocols.

- Dot blot is a simple and quantitative method, if reliable reference material is used. However, it suffers from the limited linearity and dynamic range of western blotting in general.

Given the practical drawbacks of the aforementioned techniques, a conventional sandwich ELISA currently appears to be superior in terms of inter- and intralaboratory variation as well as ease of use. Therefore, it represents the best format for reliable and reproducible quantification of total rAAV capsid titers.

Use of shuffled/mutated AAV:

The recognition of shuffled/mutated AAV vectors depend on the specific capsid region which is affected by the shuffling/mutation. The capture antibodies used for PROGENs AAV ELISAs bind specific and, in some cases, well defined conformational epitopes. These epitopes are generated by the capsid assembly of the corresponding AAV serotypes. A first indication that the ELISA might recognize your shuffled/mutated AAV vector is the presence of the antibody-binding epitope. However, changes in the protein sequences of the capsid proteins might also influence conformation of the proteins, hence the conformation of the epitopes presented on the AAV capsid. This might influence binding affinity of the antibody and affect determination of the titer based on the (non-shuffled) Kit Control provided with the AAV ELISA kit. Since these properties strongly depend on the specific shuffling/mutation performed, PROGEN cannot guarantee successful and precise quantification of your shuffled/mutated AAV vector. Even if the antibody binding epitopes are still present on your shuffled/mutated AAV capsid, your assay needs to be tested and optimized for your specific AAV vector.

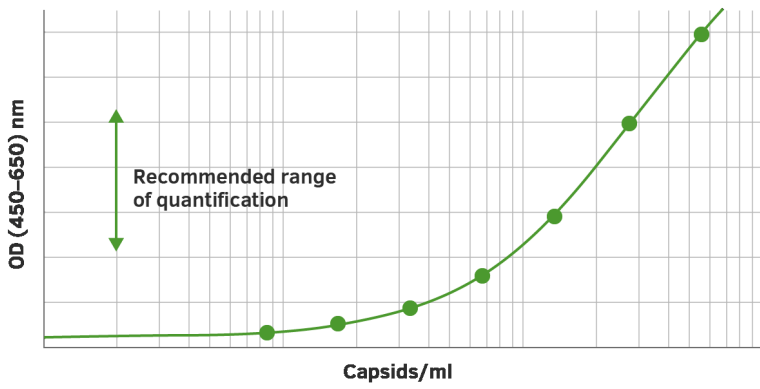
PROGEN highly recommends the production and calibration of a suitable (shuffled/mutated) Kit Control, to ensure reliable titer determination of your individually shuffled/mutated AAV vector with PROGEN ELISA kits.

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Product images



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Table 1: Analysis of matrix effects of different additives on the AAV8 Titration ELISA

Additive / Matrix*	PRAAV8 tolerated concentration ($\Delta < 10\%$)**
Pluronic® F-68	0.1 %
MgCl ₂	50 mM
Triton™ X-100	0.5 %
Deoxycholate	0.25 %
TWEEN® 20	0.5 %
EDTA	10 mM
NaCl	1 M
Iodixanol	0.16 %
HEK293 cell lysate	0.625 µg/µl
Insect cell lysate	0.625 µg/µl

*Additives were added in different concentrations to purified AAV preparations of the corresponding AAV serotypes.
 ** The tolerated concentration was defined as the highest concentration giving less than 10% deviation of the defined AAV capsid titer for the corresponding serotype.

Analysis of matrix effects of different additives on the AAV8 Titration ELISA.

References

Publication	Species	Application
Chung, S.H. et al. Host immune responses after suprachoroidal delivery of AAV8 in nonhuman primate eyes. Hum Gene Ther. 32, 682-693(2021).		
François, A. et al. Accurate Titration of Infectious AAV Particles Requires Measurement of Biologically Active Vector Genomes and Suitable Controls. Mol. Ther. - Methods Clin. Dev. 10, 223â€"236 (2018).		

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