

Product datasheet

AAV5 Xpress ELISA

Short overview

Cat. No.	PRAAV5XP
Quantity	96 tests

Product description

Application	ELISA
Product description	Unique microtiter plate enzyme immunoassay for the quantitation of intact filled and empty AAV5 capsids. The capture antibody detects a conformational epitope not present on unassembled capsid proteins
Kit control/Standard	AAV5 empty capsids; lyophilized
Form supplied	Reagent kit, 12 x 8-well strips
Storage	2-8°C
Intended use	Research use only
Application	ELISA
Reactivity	AAV5

Background

Fast, easy and reliable AAV5 titer determination with PROGEN's AAV5 Xpress ELISA:

- assay time was reduced to less than 2 hours by adjustment of the kit components and the protocol of our standard AAV5 Titration ELISA
- seamless and risk-free transition from our standard AAV5 Titration ELISA (PRAAV5) to the Xpress version since the composition of the kits core characteristics are not changed
- same accuracy and reproducibility as the standard AAV5 Titration ELISAs

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

Table 1: Analysis of matrix effects of different additives on the AAV5 Xpress ELISA

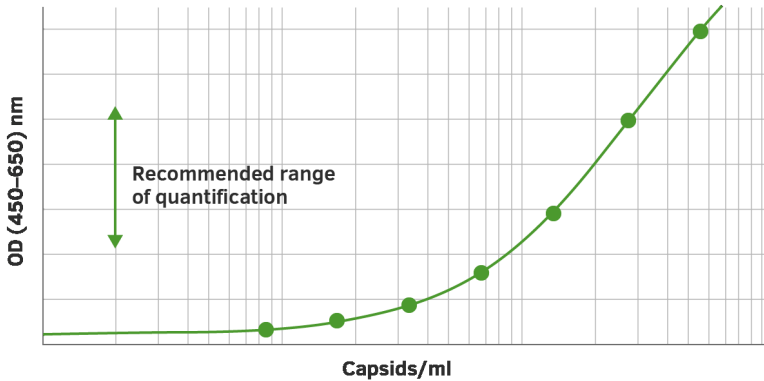
Additive / Matrix*	PRAAV5XP tolerated concentration (Δ<10%)**
Pluronic® F-68	0.1%
MgCl ₂	50 mM
Triton™ X-100	0.5%
Deoxycholate	0.5%
TWEEN® 20	0.5%
EDTA	10 mM
NaCl	20 mM
Iodixanol	0.3%
HEK293 cell lysate	1.25 µg/µl
Insect cell lysate	2.5 µg/µl

*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 AAV5 Xpress ELISA.



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