Gyrolab® AAVX Titer Kit

Product Information Sheet D0036870/E

The generic Gyrolab® AAVX Titer Kit in combination with Gyrolab system quickly delivers high quality titer data for AAV serotypes 1–8 and AAVrh10 using smaller sample volumes and with better overall performance compared to ELISA.

- Fast results for data-driven decisions – 96 data points in 80 minutes
- Improved assay working range - less need for dilutions and repeats
- Save precious samples - reproducible data from 10x less sample volumes
- Easy to use – plug and play
- Increased sample throughput – up to 960 data points in one working day
  - Automated analyses – fewer manual operations
  - Broad dynamic range – fewer dilutions

Introduction

Vectors based on the nonpathogenic adenovirus-associated virus (AAV) have become highly valuable in gene therapy. Recombinant AAV can attach to and enter the target cell, transfer to the nucleus and persist as an episomal form, expressing the transgene in a stable manner. Different AAV serotypes target different organs and tissues. Commonly used serotypes include AAV2, which has a natural tropism towards skeletal muscles, neurons, vascular smooth muscle cells, and hepatocytes and has been used for cancer treatment, and AAV8, which is a robust vector for gene delivery to liver, skeletal muscle and heart.

Vector production is an expensive process that results in a small volume of highly valuable product — for example 200 L of bioreactor product concentrated down to 20 mL. This means that analytical methods that require only a small volume of sample are at a premium. To meet this need, Gyros Protein Technologies has developed Gyrolab AAVX Titer Kit to quickly deliver high quality titer data from sample volumes of less than 10 µL. Gyrolab AAVX Titer Kit data compare well with results from ELISA and extends the analytical range, reducing the need for repeat testing. (Figure 1). The AAVX ligand included in Gyrolab AAVX Titer kit is based on the highly selective affinity ligands developed with the CaptureSelect™ technology from Thermo Fisher. These ligands are also the basis of POROS™ CaptureSelect™ AAVX Affinity Resin, which is frequently used to purify AAV viral vectors. The AAVX ligand binds with high affinity and selectivity to native and recombinant AAV particles of various serotypes.

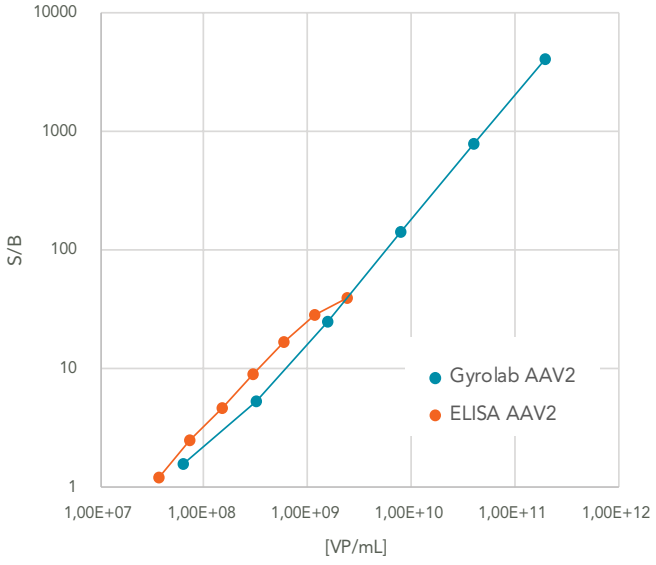

Figure 1. Gyrolab AAVX Kit gives comparable data to ELISA and extends the analytical range (this example shows data for serotype AAV2). S/B, Signal/Background.

GYROS PROTEIN Technologies

The assay

Gyrolab AAVX Titer Kit has been developed to quantify the level of AAV viral particles in manufacturing processes for cell and gene therapies. The kit is based on a sandwich assay that uses a biotinylated capture molecule and a detection molecule labeled with Alexa Fluor® 647. Gyrolab AAVX Titer Kit does not include standards and it is recommended that capsids that most resemble the vector of interest are used as a standard.

The kit contains ready-to-use capture- and detection reagents that are pre-labeled with biotin and Alexa Fluor® 647, respectively. The biotinylated anti-AAVX[1] ligand is automatically introduced into a microstructure in Gyrolab Bioaffy™ 1000 CD and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing AAV viral particles are introduced into the microstructures and captured by the immobilized anti-AAVX ligand. Bound AAV viral particles are then detected using the fluorophore-labeled anti-AAVX detection ligand (Figure 2). Results are evaluated using Gyrolab Evaluator or exported to a LIMS. All Gyrolab software programs are designed for 21 CFR part 11 compliance, ensuring that assays can be developed and transferred in regulated environments.

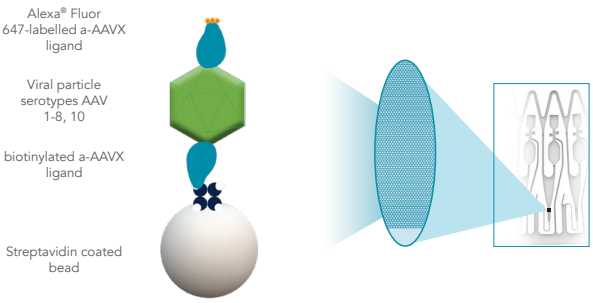

Figure 2. Sandwich immunoassay format on a Gyrolab Bioaffy 1000 CD

Assay performance

Gyrolab AAVX Titer Kit demonstrates broad, three-log working ranges for AAV serotypes 1–8 and AAVrh10 (Figure 3 and Table 1). The analytical range is comparable for serotypes 1–7 and AAVrh10. The AAVX ligand has lower affinity for AAV8, thus the assay range for serotype 8 is shifted approximately 1 log upwards. The specific working range of the assay should be established for each vector that is to be quantified and may vary between serotypes. Gyrolab AAVX Titer Kit is not compatible with AAV9.

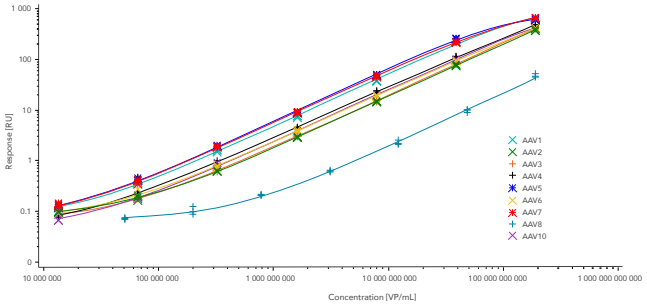

Figure 3. Overlay plot of standard curves for AAV serotypes 1–8, and AAVrh10.

Table 2 shows an example of inter- and intra-assay precision and accuracy data determined by seven QC samples of AAV2 serotype over the assay working range.

Table 1. Typical assay working ranges

Serotype	Typical Working Range VP/mL
AAV1-AAV7 and AAVrh10	1E+08 – 1E+11
AAV8	1E+09 – 1E+12

Table 2. Representative accuracy and precision data for seven QC samples for determination of assay working range (AAV2)

Sample	Expected Conc (VP/mL)	Average measured conc. (VP/mL)	Intra-run CV (%)	Inter-run CV (%)	Average Accuracy (%)	Average TE (%)
ULOQ 1	1.87E+11	1.86E+11	1.67	2.8	99.3	0.93–8.16
ULOQ 2	1.26E+11	1.22E+11	2.52	5.3	96.6	2.47–12.24
MQC	9.16E+09	9.27E+09	4.03	4.1	101.2	4.80–12.92
LQC	4.65E+08	4.56E+08	2.49	2.3	98.0	2.30–9.79
LLOQ 1	1.76E+08	1.74E+08	7.91	4.5	98.7	6.55–17.86
LLOQ 2	1.22E+08	1.20E+08	9.15	10.9	98.3	13.12–27.22
LLOQ 3	9.85E+07	8.94E+07	1.18	5.5	90.8	9.30–39.94

[1]The kit is made with Thermo Scientific™ CaptureSelect™ Biotin anti-AAVX and/or CaptureSelect™ AAV trimer/AF647 from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

Sample quantification using Gyrolab AAVX Titer Kit

Gyrolab AAVX Titer Kit was used to generate a calibration curve for rAAV5 (Figure 4), which demonstrates good linearity across three logs (~1.0E+08 VP/mL to ~1.0E+11 VP/mL). Precision was high, with replicate response CVs for duplicates below 5% from 2.0E+08 VP/mL to 1.0E+11 VP/mL. The assay results also demonstrated good dilutional linearity with comparable predictions for viral particle titer whether the sample was diluted 1:50 or 1:12800 (Figure 5).

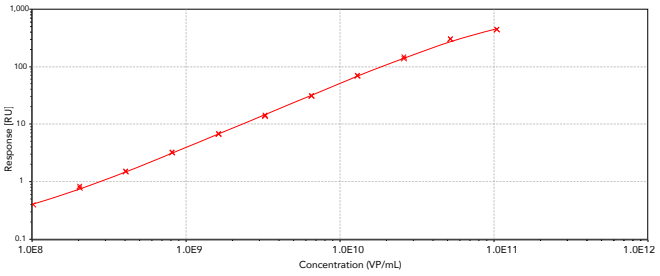

Figure 4. Calibration curve using Progen ELISA kit calibrators (PROGEN Biotechnik GmbH) and Gyrolab AAVX Kit immunoassay to predict the titer of rAAV5 samples. Data points are in duplicate.

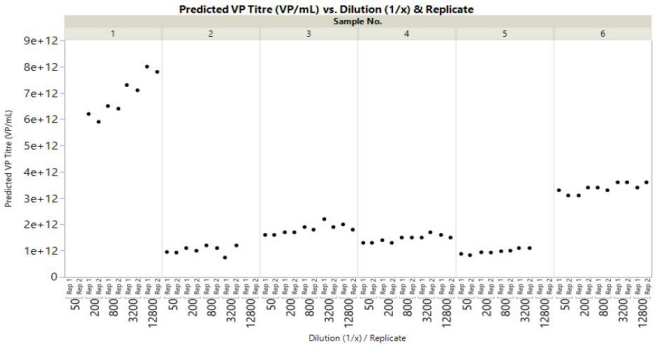

Figure 5. Predicted viral particle titer vs. sample dilution for six samples showing generally good linearity across the AAV5 assay range.

The sample quantification data in this section was generated by an industry partner.

Abbreviations used: LLOQ, lower limit of quantitation; ULOQ, upper limit of quantitation; LOD, limit of detection; CV, coefficient of variation; TE, total error; VP, viral particle.

Ordering Information

Product Number	Product name	Description
P0020695	Gyrolab AAVX Titer Kit	The kit includes 1 CD and all reagents and consumables to generate 96 datapoints.
P0020697	Gyrolab AAVX Titer CD50 Kit	The CD50 configuration is designed for high volume users and contains 50 CDs, reagents and consumables to generate 4800 datapoints.
P0020699	Gyrolab AAVX Titer Kit Sample Dilution Buffer, 25 mL	Extra sample dilution buffer.

Gyrolab AAVX Titer Kit is compatible with AAV serotypes AAV 1–8 and AAVrh10. It is not compatible with AAV9.

The following products can be also purchased separately:

P0020696	Gyrolab AAVX Titer Kit Reagents*	Includes capture and detect reagents, sample dilution and wash buffer to generate 96 datapoints (1 CD).
P0004253	Gyrolab Bioaffy 1000 CD	1000 nL sample volume, 96 datapoints. Provides extended sensitivity for e.g., PK or biomarker assays.
P0020863	Gyrolab Accessories Bundle	Includes: 96-well plate, 0.2 mL skirted PCR plates (x3), Microplate Foils (x3), Wash Station Solution 2: Gyrolab Wash Buffer pH 11(x1).

*Gyrolab AAVX Titer Kit Reagents contain ready-to-use reagents (capture and detection) for 1 CD run (96 data points). While the kit reagents have been optimized to be used in combination with Gyrolab® Bioaffy™ 1000 CD, other Gyrolab CD types can be used to customize the technical performance to meet the needs of the application.

Gyrolab AAVX Kit Contents

Each kit contains reagents and consumables for one (1) or fifty (50) CDs, for generation of 96 or 4800 data points respectively:

- Gyrolab AAVX Titer Kit Reagents (for contents, see below)
- Gyrolab Bioaffy 1000 CD
- PCR plates
- Microplate Foil
- Gyrolab Wash buffer pH 11

Gyrolab AAVX Titer Kit Reagents (P0020696)

- Capture Reagent, biotinylated anti-AAVX, ready-to-use solution
- Detection Reagent, fluorophore-labelled anti-AAVX, ready-to-use solution
- Wash buffer
- Sample Dilution Buffer

Storage conditions

Gyrolab Bioaffy 1000 CD

Refrigerate at +4 °C to +8°C, unopened package.

Shelf life (unopened package): Minimum 12 months after delivery.

Gyrolab AAVX Titer Kit Reagents

Reagents (Capture and Detect) must be stored frozen to maintain functionality.

Upon kit arrival, take reagents out of kit package and store at -20 °C.

Related products

Scan the QR-code to learn more about our other ready-to-use kits and solutions used for bioprocess analytics:

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