

# Gyrolab® AAV9 Titer Kit

Product Information Sheet

D0039753/A

Gyrolab® AAV9 Titer Kit in combination with Gyrolab system quickly delivers high quality titer data for AAV serotype 9 (AAV9).

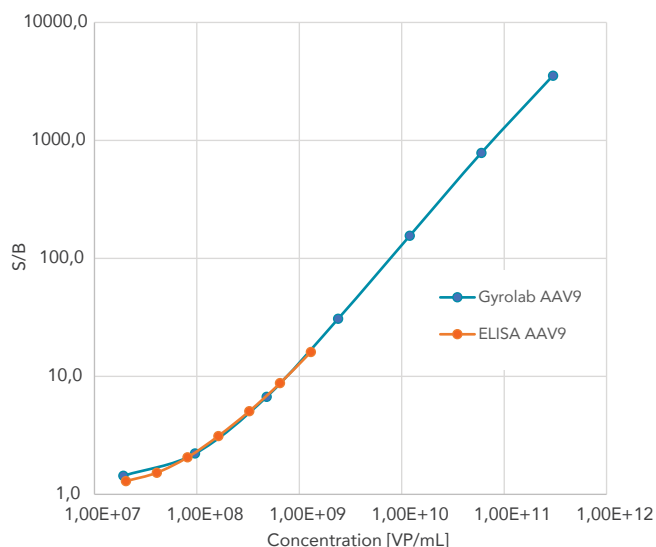
- Fast results for data-driven decisions – 96 data points in 80 minutes
- Broad assay working range – reduces need for dilutions and repeats
- Reproducible data generated from small sample volumes
- Easy to use – plug and play
- High sample throughput – up to 960 data points in one working day
  - Automated analyses – fewer manual operations
  - Broad dynamic range – fewer dilutions



## Introduction

Recombinant AAVs have become promising vectors for therapeutic gene delivery due to their nonpathogenic ability to package and express foreign genes in the absence of active cell division in a broad range of tissues. The human AAV9 serotype can be used to target cardiac and skeletal muscle, liver and pancreatic tissue, the eye, and also the central nervous system. The low prevalence of preexisting antibodies to AAV9 in humans also makes this serotype an attractive gene delivery vector.

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 AAV9 Titer Kit that, in combination with Gyrolab systems, quickly delivers high quality physical titer data from sample volumes of less than 10 µL from different stages of a bioprocess run or final purified product. The anti-AAV9 ligand included in Gyrolab AAV9 Titer kit is based on the highly selective affinity ligands developed with the CaptureSelect™ technology from Thermo Fisher Scientific. These ligands are also the basis of POROS™ CaptureSelect AAV9 Affinity Resin, which is frequently used to purify AAV9 viral vectors.

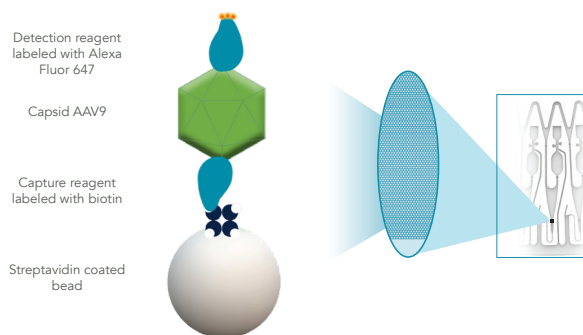


**Figure 1.** Gyrolab AAV9 Kit gives comparable data to ELISA and extends the analytical range. S/B, Signal/Background.

## The assay

Gyrolab AAV9 Titer Kit has been developed to quantify the level of AAV9 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 AAV9 Titer Kit does not include standards and it is recommended that capsids that most resemble the vector of interest are used as a standard.

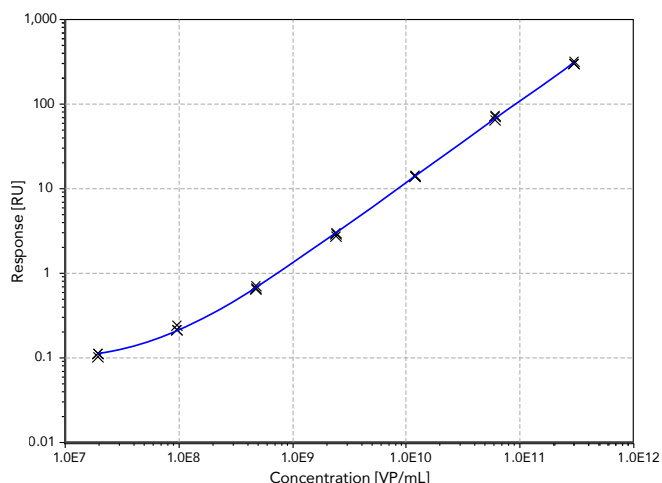
The kit contains ready-to-use capture- and detection reagents that are pre-labeled with biotin and Alexa Fluor 647, respectively. The biotinylated anti-AAV9 ligand is automatically introduced into a microstructure in Gyrolab Bioaffy™ 1000 CD and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing AAV viral particles are introduced into the microstructures and captured by the immobilized anti-AAV9 ligand. Bound AAV9 is then detected using the fluorophore-labeled anti-AAV9 detection ligand<sup>1</sup> (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 AAV9 Titer Kit demonstrates a broad, three-log working range (see Figure 3 and Table 1). The specific assay working range should be established for each vector that is to be quantified.



**Figure 3.** A typical standard curve using recombinant AAV9 empty capsid. Each standard sample was analyzed in triplicate.

**Table 1.** Typical kit working range

LLOQ (VP/mL)	ULOQ (VP/mL)
~2.0E+08	~2.0E+11

Intra- and inter-run precision were determined for five QC samples with different concentrations of AAV9 empty capsids. The data is summarized in Table 2. Disrupted or aggregated capsids may impact assay performance.

**Table 2.** Intra- and inter-run precision data for five QC samples covering the assay working range. The QC samples were run in triplicate in six separate runs on three instruments by two operators.

Sample	Conc. (VP/mL)	Average measured conc. (VP/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ	2.0E+11	2.0E+11	4.3	4.9
HQC	1.0E+11	1.0E+11	2.7	2.8
MQC	5.0E+09	4.8E+09	3.6	4.1
LQC	5.0E+08	4.8E+08	4.2	4.2
LLOQ	2.0E+08	1.9E+08	10	11

<sup>1</sup>The kit is made with Thermo Scientific™ CaptureSelect™ Biotin Anti-AAV9 Conjugate and Thermo Scientific™ CaptureSelect™ Alexa Fluor™ 647 Anti-AAV9 Conjugate. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

## Sample quantification using Gyrolab AAV9 Titer Kit

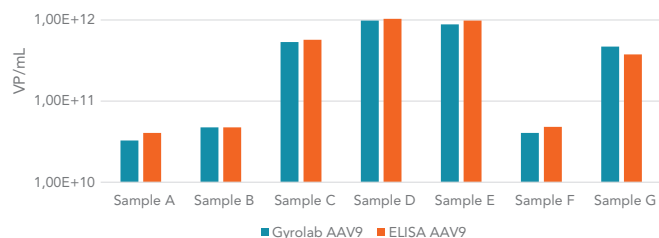
Gyrolab AAV9 Titer Kit was used to quantify seven bioprocess samples from various points in the purification process (Table 3).

**Table 3.** Quantification of several bioprocess samples in three runs, using the Gyrolab AAV9 Titer Kit.

Sample	Average conc. run 1 (VP/mL)	Average conc. run 2 (VP/mL)	Average conc. run 3 (VP/mL)	Average final conc. (VP/mL)	Total CV (%)
Sample A	3.37E+10	3.06E+10	3.40E+10	3.28E+10	5.74
Sample B	4.82E+10	4.12E+10	5.31E+10	4.75E+10	12.59
Sample C	5.19E+11	5.18E+11	5.65E+11	5.34E+11	5.03
Sample D	1.02E+12	9.44E+11	9.77E+11	9.80E+11	3.89
Sample E	9.24E+11	8.40E+11	8.93E+11	8.86E+11	4.80
Sample F	4.39E+10	4.04E+10	3.68E+10	4.04E+10	8.79
Sample G	4.82E+11	4.43E+11	4.83E+11	4.69E+11	4.86

The seven samples were also analyzed using ELISA and both assays produced comparable results (Figure 4).

**Note:** Results from ELISA and Gyrolab assays may not be comparable for all bioprocess samples due to variations in sample nature and differences in the measuring techniques.



**Figure 4.** Quantification of different bioprocess samples using the Gyrolab AAV9 Titer Kit, and comparison between Gyrolab and ELISA.

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

## Ordering Information

Product Number	Product name	Description
P0020747	Gyrolab AAV9 Titer Kit	A single CD kit that contains reagents and consumables to generate 96 data points.
P0020749	Gyrolab AAV9 Titer CD50 Kit	The CD50 configuration is designed for high volume users and contains reagents and consumables for 50 CDs. 10 reagent vials of each capture and detect contain reagents sufficient for 5 CD runs.
P0020751	Gyrolab AAV9 Titer Sample Dilution Buffer, 25 mL	Extra sample dilution buffer.

### Gyrolab AAV9 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 AAV9 Titer Kit Reagents (for contents, see below)
- Gyrolab Bioaffy 1000 CD
- PCR plates
- Microplate Foil
- Gyrolab Wash buffer pH 11

#### Gyrolab AAV9 Titer Kit Reagents

- Capture Reagent, biotinylated anti-AAV9, ready-to-use solution
- Detection Reagent, fluorophore-labeled anti-AAV9, ready-to-use solution
- Wash buffer 1 and Wash buffer 2
- 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 AAV9 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.

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### RELATED PRODUCTS

#### Gyrolab p24 Kit

*For quantification of p24 protein in manufacturing of lentiviral vectors.*

P0020659	Gyrolab p24 Kit
P0020665	Gyrolab p24 CD50
P0020664	Gyrolab p24 Standard
P0020674	Gyrolab p24 Sample Dilution Buffer

#### Gyrolab AAVX Titer Kit

*For AAV product titer determination in process development and in the manufacturing of AAV viral vectors.*

P0020695	Gyrolab AAVX Titer Kit
P0020697	Gyrolab AAVX Titer CD50 Kit
P0020699	Gyrolab AAVX Titer Kit Sample Dilution Buffer

#### HEK 293 HCP Solution for Gyrolab

*For the detection of HEK 293 host cell protein impurities in bioprocess samples.*

G650S	HEK 293 HCP Assay Reagent Set for Gyrolab (Order from Cygnus Technologies)
P0020667	Gyrolab Bioaffy 1000 HC Assay Toolbox
P0020668	Gyrolab Bioaffy 1000 HC Assay Toolbox CD50
P0020670	Gyrolab HCP Sample Dilution Buffer