GYROS PROTEIN Technologies

Gyrolab® Assay Protocol

AAV9 Titer Assay

INTRODUCTION

Adeno-associated viruses (AAV) are non-pathogenic ssDNA viruses and currently one of the most widely used vehicles for delivery of gene therapies. The virus transduces a variety of dividing and non-dividing cells showing long-term gene expression with low cellular immune response.

We have developed a three-step sandwich Gyrolab® assay to determine AAV9 titer in bioprocess samples. The assay has a broad analytical range with an approximate LOD of 1.5E7 VP/mL, LLOQ of 3.0E7 VP/mL, and ULOQ of 8.0E10 VP/mL. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity while maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a three-step sandwich assay with biotinylated anti-AAV9 monoclonal antibody as a capture molecule and the same anti-AAV9 monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule.

anti-AAV9 mAb

empty AAV9 capsids

anti-AAV9 mAb

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 4-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 3.0E7 VP/mL to 8.0E10 VP/mL (Table 1). The Limit of Detection (LOD) was determined as the concentration corresponding to at least two standards deviations above the assay blank.

The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicates in three runs, was <20% (Table 2).

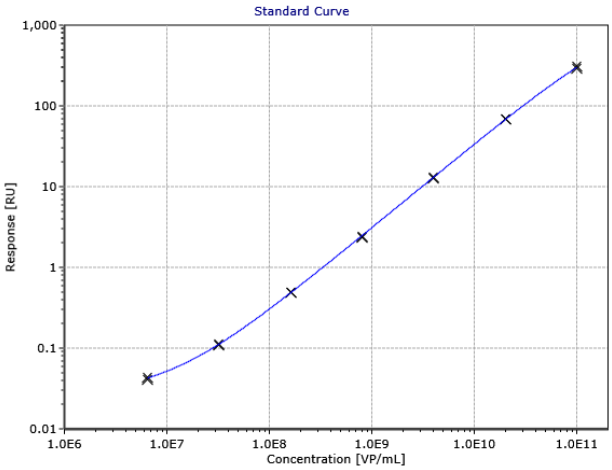

Figure 1 Typical standard curve in Rexxip® F

Table 1 Estimated Assay Range in based on three runs

LOD (VP/mL)	LLOQ (VP/mL)	ULOQ (VP/mL)
~ 1.5E7	~ 3.0E7	~ 8.0E10

Table 2 Accuracy and precision data of QC samples, n = number of runs

QC	Expected Conc (VP/mL)	Average Measured Conc (VP/mL)	Inter Run CV (%; n=3)	Average Intra Run CV (%; n=3)	Average Total Error (%; n=3)
1	3.0E7	3.18E7	7.1	5.9	12
2	6.0E7	6.46E7	4.6	3.8	11
3	3.0E9	3.02E9	4.0	2.8	5.7
4	5.0E10	5.20E10	1.5	1.4	5.3
5	8.0E10	8.18E10	1.7	1.5	3.8

Shifting the dynamic range

For higher analyte concentrations, Gyrolab Bioaffy™ 20 HC can be used as an alternative to Gyrolab Bioaffy 1000, instead of extensive dilutions of the samples. An approximate 3-log offset in dynamic range can be achieved by changing the CD type used to analyze the samples (Figure 2). For Gyrolab Bioaffy 20 HC, a three-step method with two wash solutions (20HC-3W-011-A) and a 0.1% PMT setting was used.

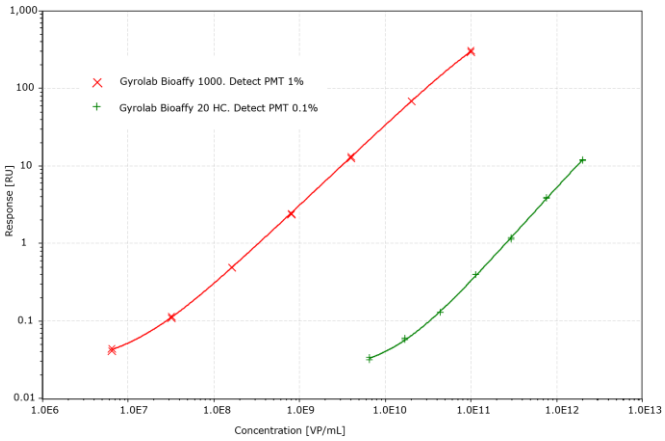

Figure 2 Standard curves in Rexxip F generated in parallel using Gyrolab Bioaffy 1000 and Gyrolab Bioaffy 20 HC

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Bioaffy 1000 CD. The assay was set up using a three-step method with two wash-solutions (1000-3W-006-A) and a 1% PMT setting. The assay buffer was Rexxip F. Mouse monoclonal anti-AAV9 antibody (clone ADK9-1R) from PROGEN was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL, diluted with PBS-T.

The detection antibody, labeled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the mouse monoclonal anti-AAV9 antibody (clone ADK9-1R) from PROGEN, diluted to 25 nM in Rexxip F. The assay standard used was AAV9 empty capsids from Sirion Biotech. The standard and controls were prepared in Rexxip F.

Summary table

Capture	Mouse monoclonal anti-AAV9 antibody (clone ADK9-1R, PROGEN Biotechnik GmbH) ordered without BSA as a bulk product request, biotinylated and diluted to 100 µg/mL in PBS-T
Detection	Mouse monoclonal anti-AAV9 antibody (clone ADK9-1R, PROGEN Biotechnik GmbH) ordered without BSA as a bulk product request, labeled with Alexa Fluor 647 diluted to 25 nM in Rexxip F
Analyte	AAV9 empty capsids (custom made AAV9 empty capsids, Sirion Biotech) in Rexxip F
CD-type	Gyrolab Bioaffy 1000
Method	1000-3W-006-A
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11
PMT-setting	1%
Expected dynamic range	Approximately 3.0E7-8.0E10 VP/mL

Recommendations

When developing this assay for a specific bioprocess, it is important to screen matrices and assess backgrounds. Parameters, such as LLOQ, should be validated in-house. Data given in this document should only be considered as guidance.

For additional support contact your local Field Application Support