Gyrolab® Anti-AAVX Kit

Enables detection of anti-AAV antibodies in human and cynomolgus monkey matrices

Product Information Sheet D0048560/C

- Accelerate assessment of total binding anti-AAV antibodies in cyno and human samples
- Eliminate the need for AAV serotype-specific assay development
- Benefit from ready-to-use format that offers fast and reliable results, reducing variability and manual errors
- Automate total binding anti-AAV antibodies detection with a 90 minute run time and minimal capsid consumption

Introduction

Screening for pre-existing antibodies against adeno-associated virus (AAV) is vital in AAV vector-based gene therapy development as it helps predict potential immune reactions, tailor therapeutic strategies to individual patients, and refine clinical trial designs allowing for better patient stratification and selection. This approach enhances treatment safety and efficacy while ensuring more accurate interpretation of treatment outcomes, thereby advancing the development and implementation of gene therapy interventions. Moreover, in preclinical studies, animals must also undergo screening for pre-existing anti-AAV antibodies for selection between and within species.

Screening methods for anti-AAV antibodies have seen significant development, primarily through cell-based transduction inhibition (TI) assays, known as NAb assays, and total antibody (TAb) assays, also referred to as binding antibody assays. However, TAb methods have notable limitations. Firstly, they lack comprehensive serotype coverage, requiring labor-intensive development of serotype-specific assays. Secondly, the need for capsid labeling introduces variability and consumes time and resources. Additionally, these methods often demand larger quantities of viral capsids, limiting batches for commercialization. These challenges impact assay variability, time consumption, resource allocation, and commercial efficiency.

In response, Gyros Protein Technologies has developed an innovative Anti-AAVX kit that allows for the detection of binding anti-AAV antibodies against most commonly used AAV serotypes without the need for developing serotype-specific assays, thereby saving time and resources. Moreover, it eliminates the requirement for labeling capsids, substantially enhancing data robustness by eliminating labeling batch-to-batch variation. Gyrolab® Anti-AAVX Kit includes Gyrolab

Gyrolab Anti-AAVX Kit streamlines the assessment of pre-existing antibodies against AAV vectors, enabling detection of total anti-capsid antibodies in cyno and human samples.

- Generic format speeds up analysis by avoiding the need for multiple AAV serotype-specific assays.
- Ready-to-use assay provides convenience and rapid results, eliminating cross-capsid optimization.
- Enhanced data reliability is attained by omitting capsid labeling, reducing variability and manual errors.
- Automated microfluidic Gyrolab assay with minimal capsid consumption, maximizing drug availability for commercialization.

Anti-AAVX Kit Reagents (capture and detection) along with all necessary consumables, such as discs, buffers, a positive control and accessories, sufficient for five microfluidic disc runs (480 data points). Although the kit is supplied with a Gyrolab Bioaffy™ 1000 disc, alternative Gyrolab disc types can be utilized with the Gyrolab Anti-AAVX Kit Reagents to tailor technical performance according to application requirements. Gyrolab Anti-AAVX Kit is for research use only and are not intended for diagnostic use.

GYROS PROTEIN Technologies

Assay principle

Gyrolab Anti-AAVX Kit is based on the well-established sandwich principles with two capture reagents (4-step assay). The biotinylated capture reagent is automatically introduced into a microstructure in the Gyrolab Bioaffy microfluidic disc and captured on streptavidin-coated beads in the flow-through affinity column. The second capture reagent: an AAV capsid of choice, is introduced and interacts with the first capture reagent forming a complex. Subsequently, samples being screened for anti-AAV antibodies are volume defined and introduced into the microstructures where anti-AAV antibodies are captured in the capture column. Finally, a detecting reagent labeled with Alexa Fluor™ 647 is added. The integrated fluorescent signal represents the response from the reaction of the anti-AAV antibody to the AAV capsid. 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.

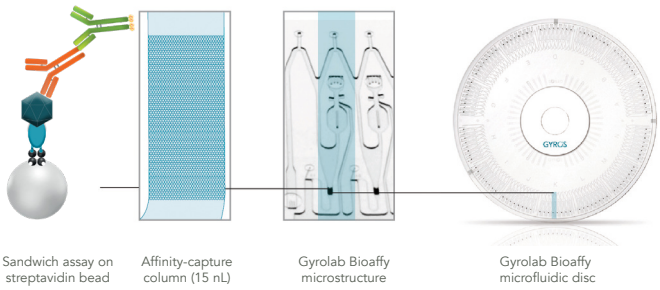

Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy 1000

Assay performance

Assay set up & data analysis

The assay setup utilized consists of two distinct experiments. In the first experiment, the ready-to-use capture reagent from the Gyrolab Anti-AAVX Kit Reagents is employed as the initial capture reagent, followed by the AAV capsid of interest as the secondary capture (spiked samples). The second experiment also utilizes the ready-to-use capture reagent from the Gyrolab Anti-AAVX Kit Reagents as the initial capture reagent, but in this case, Rexxip® H is added as the secondary capture (unspiked samples).

This is to allow for calculation of individual signal inhibition for each sample according to the method below:

- signal ratio = mean response $_{unspiked}$ / mean response $_{spiked}$
- % signal inhibition = 100 * (1 - signal ratio)

Samples with a % signal inhibition ≥ 30 and ≤ -30 are excluded from the following calculations:

Calculation of mean signal ratio of all samples followed by calculation of the ratio standard deviation of the signal ratios of all samples.

Determination of cut point to apply to the previously calculated % signal inhibition:

- confirmatory ratio = mean signal ratio - (3.09 * ratio standard deviation)
- cut point (%) = 100 * (1 - confirmatory ratio)

Samples with a % signal inhibition equal to or above the calculated cut point are qualified as positive.

Accuracy and precision

Intra- and inter- run precision were determined for human and cyno samples. Three QC samples of each species previously assessed to exhibit high, medium, or low responses to the specific serotype under assessment, were diluted in Rexxip H and subjected to assay. The data for human samples assessed against AAV2 is summarized in Table 1, cyno samples assessed against AAV2 and AAV8 are summarized in Table 2 and 3 respectively. All data shows high intra- and inter-run accuracy and precision.

Table 1. Intra- and inter-run precision data for QC samples covering the assay working range. Human serum samples assessed against AAV2. The QC samples were run in triplicate in four separate runs over more than two days.

Human AAV2		
Sample	Intra run %CV (n=4)	Inter-run %CV (n=4)
High	0.5-3	2.4
Medium	1-3.8	9
Low	2-4.8	5.2

Table 2. Intra- and inter-run precision data for QC samples covering the assay working range. Cyno samples assessed against AAV2. The QC samples were run in triplicate in four separate runs over more than two days.

Cyno AAV2		
Sample	Intra run %CV (n=4)	Inter-run %CV (n=4)
High	0.7-2.2	7.3
Medium	1-6.2	8.4
Low	1.6-9	11.4

Table 3. Intra- and inter-run precision data for QC samples covering the assay working range. Cyno samples assessed against AAV8. The QC samples were run in triplicate in four separate runs over more than two days.

Cyno AAV8		
Sample	Intra run %CV (n=4)	Inter-run %CV (n=4)
High	1.3-7.5	4.8
Medium	3.2-7.4	6.8
Low	0.2-6.4	8.6

Assay genericity

Assay genericity i.e. the ability of the assay to accurately detect AAV vectors regardless of serotype, was assessed by testing for pre-existing TAbs against seven commonly employed AAV serotypes (AAV1, AAV2, AAV3, AAV5, AAV6, AAV8 and AAV9) for a cohort of 30 human serum samples.

5/30 samples exhibited indiscriminate positivity for all tested serotypes, while 15/30 samples showed indiscriminate negativity. AAV1, AAV2, and AAV3 had the highest number of positive individuals, whereas AAV5 and AAV9 had the lowest. These findings align with established data on the prevalence of AAV serotypes in humans.

Table 4. Human serum sample genericity. Strong positive samples marked in green, weaker positive samples are highlighted in pale yellow. Negative samples remain uncolored. Number of runs (n) per serotype as below n=1, 6, 1, 6, 2, 2, 4 for AAV1, AAV2, AAV3, AAV5, AAV6, AAV8 and AAV9 respectively.

Human serum samples	AAV1	AAV2	AAV3	AAV5	AAV6	AAV8	AAV9
	signal inhibition (%)						
1	-7	-11	-5	-31	-16	-7	-2
2	-8	-10	-2	-28	-14	0	-5
3	60	39	53	27	29	21	5
4	-16	-10	-3	-32	-25	-12	-3
5	57	6	23	-5	46	38	8
6	13	-4	1	-2	0	-23	-10
7	-21	-8	-9	-20	-20	-11	8
8	-5	14	8	-27	-9	-6	-1
9	66	53	61	-1	45	14	4
10	99	99	99	99	99	99	98
11	26	55	42	-8	16	14	6
12	45	54	57	7	42	32	15
13	79	62	82	74	59	71	32
14	13	2	0	-27	0	-19	3
15	-2	-18	-4	-37	-16	-16	2
16	0	5	8	-30	-10	-3	5
17	-10	-7	8	-23	-16	-6	-5
18	51	53	69	2	36	26	3
19	-4	-5	8	-18	-11	-6	2
20	5	12	18	-14	-2	0	-2
21	34	82	39	19	38	36	2
22	40	50	65	22	29	30	12
23	98	95	98	98	94	89	13
24	39	30	38	7	25	11	-13
25	-7	-7	-14	-32	-6	-6	-4
26	-14	-2	-9	-37	-7	-6	0
27	-12	-16	-83	13	-13	-9	-10
28	95	95	96	86	95	95	79
29	48	73	56	20	23	37	18
30	64	88	66	78	72	80	49

The same assessment was performed for a cynomolgus monkey cohort of 14 samples; 1/14 samples were indiscriminately positive for all serotypes, while 3/14 samples were indiscriminately negative. AAV8 and AAV9 had the greatest number of positive individuals, while AAV5 had the lowest (data not shown). This finding aligns with existing data regarding the prevalence of AAV serotypes in non-human primates. The distribution of positive qualification for various serotypes among different individuals, without uniform readings for all serotypes in all samples, suggests that the assay is indeed generic and capable of successfully discriminating between serotypes.

Comparison vs independent NAb and TAb assays

To confirm the robustness of the kit to screen for binding antibodies against AVV vectors for different serotypes, a comparison was performed with independent NAb and TAb assays.

NAb assays were completed using SVAR Life Science iLite AAV Neutralizing Platform for 2 different serotypes, i.e. AAV2 and AAV8 (results not shown), whereas SVAR Life Science ELISA TAb assay was performed for serotype AAV2.

The NAb results for 12 human serum samples are given in % neutralization (transduction inhibition). For this comparison, a cut point was set at 30% for AAV2, with samples reading above qualifying as positive and samples reading below qualifying as negative. Samples 10 and 12 came in as negative in the NAb assay and positive in the Gyrolab assay while 9 qualified as positive in the NAb and negative in the Gyrolab assay. There is a clear correlation between samples qualifying as positive in both assays. This speaks to the conclusion that the differences in qualification for these samples are due to the assay formats (NAb vs TAb) rather than an inaccurate read. Furthermore, the SVAR TAb assay agrees with the Gyrolab assay for all samples except for sample 3 where it registers as a weak positive in the TAb and negative in the Gyrolab assay.

Table 5. Human serum sample genericity. Strong positive samples marked in green, weaker positive samples are highlighted in pale yellow. Negative samples remain uncolored.

Method	Anti-AAVX Kit	TAb	NAb
Human serum samples	signal inhibition (%)	OD (450-620 nm)	signal inhibition (%)
1	-14.2	0.23	-5
2	-15.7	0.14	11
3	11.5	0.41	18
4	99.4	3.61	100
5	56.4	1.35	63
6	51.8	0.9	42
7	-11.9	0.19	8
8	47.9	0.75	35
9	-9.6	0.16	46
10	48.9	0.44	13
11	78.3	3.62	97
12	23.5	0.44	-22

Ordering Information

Product Number	Product name	Description
P0021063B	Gyrolab Anti-AAVX Kit	Includes capture and detect reagents, discs, positive control, sample dilution buffer, wash buffer and accessories required to generate 480 datapoints (5 discs)

Gyrolab Anti-AAVX Kit Contents

Product Number	Product name	Quantity	Description
P0021043B	Gyrolab Anti-AAVX Kit Reagents	1	Includes ready-to-use capture and detection reagents required to generate 480 datapoints (5 discs)
P0004253	Gyrolab Bioaffy 1000	5	1000 nL sample volume, 96 datapoints
P0004822	Rexxip H	1	25 mL, 1 x standard formulation, agents to neutralize heterophilic antibodies. For samples containing heterophilic antibodies (e.g., human or cyno samples)
P0021050B	Gyrolab Anti-AAVX Positive Control*	1	50 µL, contains human IgG reactive toward several AAV capsid serotypes
P0021067	Gyrolab Anti-AAV5 Positive Control	1	50 µL, contains human IgG reactive toward AAV5 capsid serotype
P0021035	Gyrolab Assay Wash Buffer 1	3	2 mL, wash buffer
Additional Gyrolab consumables			Contains 96-well plate: 0.2 mL skirted PCR plates (x15), microplate foils (x15), Gyrolab Wash Buffer pH 11 (x5)

*Used to detect capsids of serotypes AAV2, AAV3, AAV8 and AAV9. For use with AAV5 serotype, the Gyrolab Anti-AAV5 Positive Control (P0021067) can be ordered instead. (only available as stand-alone).

All products above can also be purchased separately as stand-alone products.

Gyrolab Gyrolab Anti-AAVX Kit Reagents content

Each Kit Reagents product contains ready-to-use capture and detection reagents for generation of 480 data points:

Capture Reagent1: CaptureSelect™ Biotin Anti-AAVX, ready-to-use solution, 250 µL

Detection Reagent: Alexa Fluor™ labeled anti-human IgG, ready-to-use solution, 250 µL

[1]Made with Thermo Scientific™ CaptureSelect™ AAVX Biotin anti-AAVX from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries"

Storage conditions

Gyrolab Anti-AAVX Kit Reagents are shipped refrigerated and upon arrival should be stored at -20°C.

Once rethawed, the reagents can be stored up to one week at +4 to +8°C.

Supplementary components contained in the Gyrolab Anti-AAVX Kit should be stored as stated on individual labels.

Related products

Scan the QR-code to learn more about our other ready-to-use kits and solutions in cell and gene therapy development.

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