ELISA Kit performance summary: Accurate detection of AVIPure® HiPer™ AAV8 Affinity Ligand in the presence of AAV8 Capsids

Performance Summary

Introduction

The AVIPure® HiPer™ AAV8 Affinity Ligand ELISA Kit (part number 9-EL-0110) from Repligen Corporation provides accurate and precise quantitation of residual ligand in AAV8 samples purified with resin manufactured with AVIPure HiPer AAV8 Affinity Ligand.

Note: *This kit is not for use with samples purified using agarose AVIPure AAV8 affinity resin, which should instead be analyzed using Cygnus kit P/N F1005.*

Industry requirements dictate that residual ligand must be quantified. Quantitation with ELISA can be used to confirm the level of leached ligand is within the desired specification for therapeutic products. Testing for residual AAV8 Affinity Ligand may occur in several phases of development and commercial manufacturing:

- Process development: leaching characteristics of the resin under specific conditions
- Manufacturing: elution samples at critical steps within the purification process
- Final product release: document process contaminant levels and lot-to-lot consistency

ELISA Kits from Repligen are validated to ensure accurate and reproducible quantitation of residual ligand. All kits are evaluated for the following parameters:

- Intra-assay precision: ensures well-to-well reproducibility within the same plate
- Inter-assay precision: ensures plate-to-plate reproducibility
- Accuracy: demonstrates analyte detection is not affected by differences in sample matrix (e.g., cell culture media, buffer solution), measured as % recovery
- Limit of detection (LOD): lowest quantity or concentration of an analyte that can be reliably distinguished from background
- Limit of quantitation (LOQ): lowest concentration at which an analyte can be quantified

The performance of AVIPure HiPer AAV8 Affinity Ligand ELISA Kit was evaluated by detecting the AAV8 Affinity Ligand in the presence of AAV8 matrix relative to a reference standard of the same ligand in the absence of AAV8 matrix. Kit performance was evaluated for the parameters outlined above by running three (3) independent ELISA assays according to the standard protocol. Serial dilution of the ligand standard was evaluated in triplicate for each of three (3) independent assays. Subsequently, kit performance was evaluated in the presence of AAV8 matrix. All spiked samples by Dilute and Go had an initial AAV8 matrix concentration of 5x1011 vp/mL for Dilute & Go method and 2x1012 vp/mL for Boil & Boost method.

Data supports the use of the AAV8 Affinity Ligand ELISA Kit for the quantitation of AAV8 leachate in AAV8-containing samples (Table 1).

Table 1. Data Summary

Parameter	Dilute & Go	Boil & Boost
Intra-assay precision	<5%	<12%
Inter-assay precision	<14%	<14%
LoQ	0.30 ng/mL	0.25 ng/mL
Recovery	80.7 – 110%	90 – 122%
LOD	0.23 ng/mL	0.17 ng/mL

Results from Spike Recovery by Dilute & Go Method

Data Handling

Back-calculated concentration from spiked samples by Dilute & Go method were used to obtain recovery after subtracting the non-spiked AAV8 matrix background, then dividing it by theoretical concentration. AAV8 Affinity Ligand standards on the same plate were also analyzed for precision and accuracy.

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Intra-assay Precision

Average percent coefficient of variation (CV) data for standards without AAV8 matrix (Table 2) and samples with AAV8 matrix (Table 3) demonstrate intra-assay reproducibility. For standards without AAV8 matrix, average %CV ranged from 0.3 – 3.4%. For samples with AAV8 matrix, average %CV values ranged from 2.8 – 4.4% at above LOQ level.

Table 2. Intra-assay Precision and Accuracy for AAV8 Affinity Ligand Standards without AAV8 Matrix (Dilute & Go)

Theoretical Concentration (ng/mL)	Average Absorbance (A450)	Average Calculated Concentration (ng/mL)	Average Standard Deviation	Average %CV
0.3125	0.160	0.29	0.005	3.4%
0.625	0.253	0.61	0.008	3.1%
1.25	0.435	1.26	0.005	1.2%
2.5	0.740	2.46	0.002	0.3%
5	1.272	5.05	0.025	1.9%
10	1.810	10.00	0.020	1.1%

Table 3. Intra-Assay Precision for Spiked Levels of AAV8 Affinity Ligand with AAV8 Matrix (Dilute & Go)

Theoretical Concentration (ng/mL)	Average Absorbance (A450)	Average Calculated Concentration (ng/mL)	Average Standard Deviation	Average %CV
1.25	0.476	1.126	0.049	4.4%
2.5	0.784	2.372	0.066	2.8%
5	1.196	4.342	0.155	3.6%
10	1.744	8.651	0.269	3.2%

Inter-assay Precision

Average %CV data for samples with AAV8 matrix (Table 4) demonstrate inter-assay reproducibility. Across all spiked concentrations evaluated, %CV was <15%.

Table 4. Inter-assay Precision for Three Levels of AAV8 Affinity Ligand with AAV8 Matrix (Dilute & Go)

Theoretical Concentration (ng/mL)	Average Absorbance (A450)	Average Calculated Concentration (ng/mL)	Average Standard Deviation	Average %CV
1.25	0.476	1.126	0.055	4.9%
2.5	0.784	2.372	0.323	13.6%
5	1.196	4.342	0.087	2.0%
10	1.744	8.651	0.541	6.3%

Table 5. Limits of Detection (LOD) and Quantitation (LOQ) from Dilute & Go Method

Dilute & Go	ng/mL
LOD	0.23
LOQ	0.30

Accuracy

AAV8 Affinity Ligand standard curve prepared in pH 4.2 sample diluent showed $R^2 = 1$ by quadratic fit (Figure 1). Precision and accuracy (as % error) met specifications. Variability between AAV8 matrix spiked sample preparations was within 15% by Dilute & Go method, and recovery met the specification of 75 – 125%.

Figure 1. AAV8 Affinity Ligand Standard Curve from Dilute & Go Plate

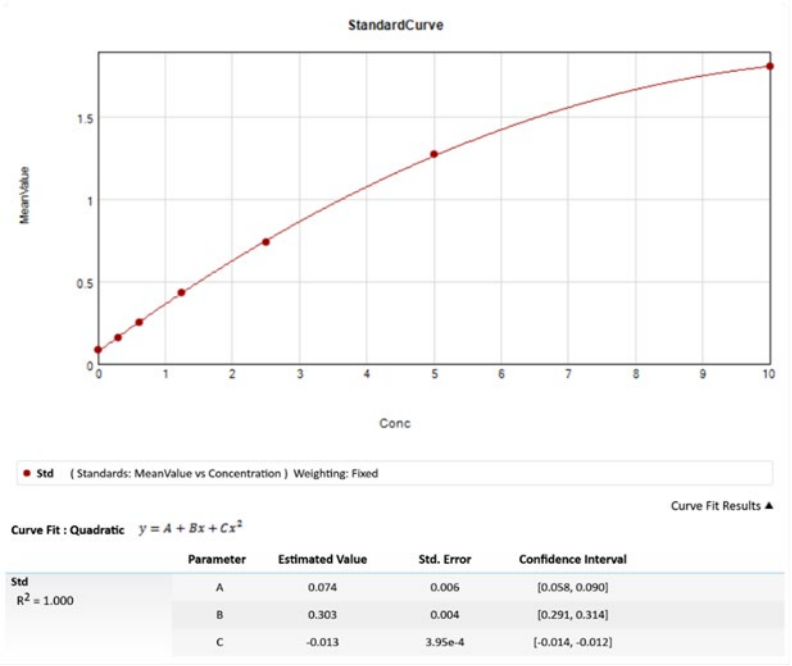


Table 6. Accuracy: Standards without AAV8 Matrix (Dilute & Go)

Concentration (ng/mL)	Relative % Error	Average % recovery
0.3125	-7.20%	92.8%
0.625	-2.45%	97.5%
1.25	0.83%	100.8%
2.5	-1.60%	98.4%
5	0.91%	100.9%
10	0.03%	100.0%

Table 7. Spike Recovery for AAV8 Affinity Ligand with AAV8 Matrix (Dilute & Go)

Concentration (ng/mL)	Relative % Error	Average % recovery
1.25	-10.1%	89.9%
2.5	-5.1%	94.9%
5	-13.2%	86.8%
10	-13.5%	86.5%

Results from Spike Recovery by Boil & Boost Method

Data Handling

Back-calculated concentration from spiked samples by Boil & Boost method were used to obtain the recovery after subtracting the non-spiked AAV8 matrix background, then dividing it by theoretical concentration. AAV8 Affinity Ligand standards on the same plate were also analyzed for precision and accuracy.

Intra-assay precision

Average percent coefficient of variation (CV) data for standards without AAV8 matrix (Table 8) and samples with AAV8 matrix (Table 9) demonstrate intra-assay reproducibility. For standards without AAV8 matrix, average %CV ranged from 3.7 to 7.8%. For samples with AAV8 matrix, average %CV values ranged from 2.8 to 11.4% at above LOQ.

Table 8. Intra-assay Precision and Accuracy for AAV8 Affinity Ligand Standards without AAV8 Matrix (Boil & Boost)

Theoretical Concentration (ng/mL)	Average Absorbance (A450)	Average Calculated Concentration (ng/mL)	Average Standard Deviation	Average %CV
0.3125	0.191	0.302	0.007	3.7%
0.625	0.293	0.631	0.023	7.8%
1.25	0.489	1.293	0.02	4.0%
2.5	0.85	2.640	0.034	4.0%
5	1.328	4.838	0.088	6.6%
10	1.924	9.954	0.144	7.5%

Table 9. Intra-assay Precision for Spiked Levels of AAV8 Affinity Ligand with AAV8 Matrix (Boil & Boost)

Theoretical Concentration (ng/mL)	Average Absorbance (A450)	Average Calculated Concentration (ng/mL)	Average Standard Deviation	Average %CV
1.25	0.693	1.414	0.044	3.2%
2.5	1.032	2.744	0.109	4.2%
5	1.393	4.599	0.504	11.4%
10	1.982	9.943	0.278	2.8%

Inter-assay precision

Average %CV data for samples with AAV8 matrix (Table 10) demonstrate inter-assay reproducibility. For samples with AAV8 matrix, %CV was <15% across all concentrations evaluated.

Table 10. Inter-assay Precision for Spiked Levels of AAV8 Affinity Ligand with AAV8 Matrix (Boil & Boost)

Theoretical Concentration (ng/mL)	Average Absorbance (A450)	Average Calculated Concentration (ng/mL)	Average Standard Deviation	Average %CV
1.25	0.693	1.414	0.051	3.6%
2.5	1.032	2.744	0.307	11.2%
5	1.393	4.599	0.488	10.6%
10	1.982	9.943	0.215	2.2%

Table 11. Limits of Detection (LOD) and Quantitation (LOQ) from Boil & Boost Method

Dilute & Go	ng/mL
LOD	0.17
LOQ	0.25

Accuracy

AAV8 Affinity Ligand standard curve prepared in sample diluent showed R^2 = 0.999 by quadratic fit (Figure 2). Precision and accuracy (as % error) met specifications. Variables between AAV8 matrix spiked sample preparation were within 15% by Boil & Boost method and recovery met the specification of 75 – 125%.

Figure 2. AAV8 Affinity Ligand Standard Curve from Boil & Boost Plate

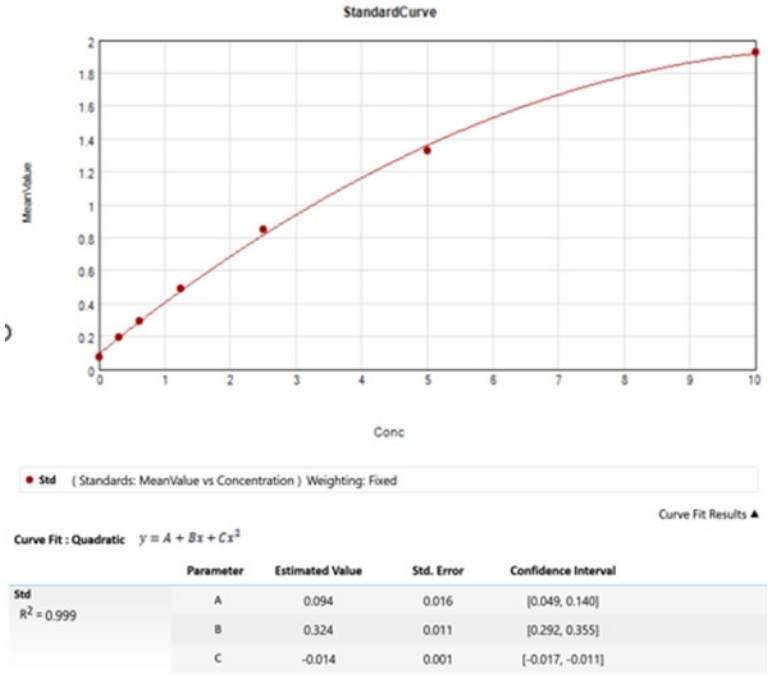


Table 12. Accuracy: Standards without AAV8 Matrix (Boil & Boost Method)

Concentration (ng/mL)	Relative % Error	Average % recovery
0.3125	-3.4%	96.6%
0.625	0.9%	100.9%
1.25	3.4%	103.4%
2.5	5.6%	105.6%
5	-3.3%	96.8%
10	-0.5%	99.5%

Table 13. Spike Recovery for AAV8 Affinity Ligand with AAV8 Matrix (Boil & Boost)

Concentration (ng/mL)	Relative % Error	Average % recovery
1.25	13.1%	113.1%
2.5	10.1%	110.1%
5	-8.0%	92.0%
10	-0.6%	99.4%

Conclusions

The performance metrics outlined demonstrate precise and sensitive detection of AAV8 Affinity Ligand using both Dilute & Boost and Boil & Boost sample preparation methods.

Intra-assay and inter-assay data indicate that precision of the three independent preparations were within acceptable parameters. Both methods had %CV values of <15% for AAV8 standards and AAV8 matrix-containing samples spiked with a range of concentrations of AAV8 Affinity Ligand.

The LOQ for the AAV8 spiked samples was 0.30 ng/mL when using Dilute & Go method and 0.25 ng/mL for Boil & Boost method. AAV8 Affinity Ligand recovery was within the accepted limit, ranging from 80.7% to 122% for samples containing AAV8 matrix.

Data support the use of AAV8 Affinity Ligand ELISA Kit for quantitation of AAV8 Affinity Ligand leachate levels in antibody-containing purification samples and drug substance.

Explanations of Calculations

Data Handling
Standard curve data points were fitted to Quadratic fit analysis. This equation allowed back-calculation of sample NGL-Impact CH1 Affinity Ligand concentrations. Recovery of each sample was calculated by dividing the observed concentration by the theoretical concentration.

Intra-assay Precision
The intra-assay precision was calculated for each NGL-Impact CH1 Affinity Ligand concentration by averaging the %CV values across all assays.

Inter-assay Precision
The inter-assay precision was calculated for each concentration point by determining the standard deviation between calculated results from each of the three assays, then dividing by the mean value.

Precision (%CV)
Precision was calculated by determining the standard deviation between each ligand concentration data point and dividing by the mean value. According to the Guidance for Industry: Bioanalytical Method Validation (May 2018), precision should be within 20% CV.

Spike Recovery
Spike Recovery was done in different levels. Recovery was represented by dividing the calculated concentration of each spiked sample using the equation from the standard curve to its theoretical concentration.

$$Recovery\ \% = \frac{Calculated\ Concentration}{Theoretical\ Concentration} \times 100$$

According to the Guidance for Industry: Bioanalytical Method Validation (May 2018), Recovery should within 20% of the actual value.

Accuracy

Accuracy is calculated by determining the percent relative error of a sample using the equation from the standard curve. The theoretical value is subtracted from the calculated value, divided by the theoretical, and reported as a percent.

$$Relative\ Error\ \% = \frac{(Calculated\ Concentration - Theoretical\ Concentration)}{Theoretical\ Concentration} \times 100$$

Accuracy of Dilution

Sample concentration was plotted versus absorbency at 450 nm. Accuracy of dilution was assessed using regression analysis with a quadratic fit.

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) was defined as 10 times the standard deviation of 0 ng/ml sample. The standard deviation of the 0 ng/ml OD value was multiplied by 10 then added to base 0 ng/ml OD value. The LOQ was then generated by entering the summed value into the standard curve. For each kit, the LOQ was reported as ng ligand per ml (ng/ml) buffer as well as ng ligand per mg IgG (ppm).

Limit of Detection (LOD)

The limit of detection (LOD) was defined as 3 times the standard deviation of 0 ng/ml spike ligand sample. The standard deviation of the 0 ng/ml OD value was multiplied by 3 then added to base 0 ng/ml OD value. The LOD was then generated by entering the summed value into the standard curve. For each kit, the LOD was reported as ng ligand per ml (ng/ml) buffer as well as ng ligand per mg IgG (ppm).

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