Version: 02
Update (MM/DD/YYYY): 01/15/2025

Product Manual

AAVX Titer Capsid ELISA Kit

- Multiple Serotypes

Cat. No. L01035

For Research Use Only. Not for Use in Diagnostic Procedures.

The operator should read technical manual carefully before using this product.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

Contents

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

I. DESCRIPTION

GenScript AAVX Titer Capsid ELISA Kit - Multiple Serotypes (Cat. No. L01035) is a Sandwich ELISA Kit designed for quantitatively measuring the total capsid titer of multiple serotypes of adeno-associated virus (AAV) in testing samples during viral vector production. The kit is universally compatible with most serotypes of AAV, providing a ready-to-use and reproducible method for quantifying different serotypes of AAV.

It is recommended to use a positive control that matches the AAV capsids in the samples to generate an accurate standard curve for quantification. Please note that this kit provides standards for AAV2 only; standards for other serotypes are not included.

AAV is now widely-used viral vectors for gene therapy. When using AAV as the viral vector in gene therapy, determination of the total capsid titer of the purified AAV particle product is an essential characterization procedure and a critical step in clinical applications.

II. ASSAY PRINCIPLE

AAVX Titer Capsid ELISA Kit - Multiple Serotypes is based on the sandwich ELISA method. When standards or samples are added to the capture plate coated with the capture antibody that recognizes multiple serotypes, the AAV capsids in the samples can be captured on the plate. The detection antibody that recognizes multiple serotypes and is conjugated with biotin, is then added to interact with the AAV capsids bound on the plate. Streptavidin-Horseradish Peroxidase conjugate is added to interact with the biotin conjugated detection antibody. After washing steps, TMB solution is added to the plate and color development is stopped by addition of stop solution. Application of the Stop Solution results in the color changing from blue to yellow. The color intensity can be read at 450 nm by a microplate reader. The AAV standard curve can be used to accurately quantify the amount of AAV capsids in the samples.

III. KIT CONTENTS

The kit provides the following reagents and items.

Component	Quantity/Size	Part No.
Capture Plate	1 plate	W1-80
AAV2 Control	2 vials (Specified on product label)	W1-10
Detection Antibody	1 bottle (12 mL)	W1-20
Streptavidin-HRP	1 bottle (12 mL)	W1-30
Sample Dilution Buffer	1 bottle (60 mL)	W1-60
10× Lysis Buffer	1 vial (1.5 mL)	W1-61
20× Wash Solution	1 bottle (60 mL)	W1-70
TMB Solution	1 bottle (12 mL)	A1-40
Stop Solution	1 bottle (6 mL)	A1-50
Plate Sealer	2 pieces	-

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

- Capture Plate: 96 well microplates (8 wells x 12 strips); 12 strips are configured in plate; plate is sealed in a foil pouch with a desiccant.
- AAV2 Control is two vials of lyophilized powder containing empty AAV2 capsids. Reconstituted with deionized or distilled water before use. The number of empty AAV2 capsids in each batch of AAV2 Control is different, and this information is specified on the product label. The reconstituted AAV2 Control can be stored at 2°C to 8°C with the kit for up to 2 weeks. For long term storage, please aliquot and store at -20°C or below. Avoid repeated freezing and thawing cycles.
- GenScript offers the 20× Wash Solution (GenScript, B00063) if customer requires additional wash solution.
- The kit does not include a paper manual. Please download the latest version of the manual from the GenScript official website.

IV. STORAGE

The unopened kit is stable for at least 12 months from the date of manufacture at 2°C to 8°C, and the opened kit is stable for up to 1 month from the date of opening at 2°C to 8°C.

V. REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

- Other AAV serotypes standards for standard curves and quality control (excluding AAV2 standard)
- Microplate reader capable of measuring absorbance at 450 nm
- Automated microplate washer
- Deionized or distilled water
- Graduated cylinder
- Plastic container
- Tubes to aliquot and dilute samples
- 10 µL, 200 µL, and 1000 µL precision pipettes
- 10 µL, 200 µL, and 1000 µL pipette tips
- Multichannel pipettes
- Disposable reagent reservoir
- Absorbent paper
- Laboratory timer
- Refrigerator

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

- Centrifuge
- Digital Thermostat Water Bath
- 37 ± 2 °C Incubator

VI. PRECAUTIONS

1. Any materials that may be contaminated with potentially infectious materials should be treated as infectious materials in accordance with local regulations. For more detailed information, please contact your local lab safety committee.
2. All personnel working with AAV must complete appropriate Biosafety Training.
3. Reagents that contain preservatives may be toxic if ingested, inhaled, or spilled on skin.
4. Avoid contact of skin, eyes, or clothing with Stop Solution or TMB Substrate. Keep container tightly closed. In case of an accident, please seek medical assistance immediately.
5. Do not use the kit if there is any visible damage to the packaging or kit contents.
6. Do not mix components from different batches. Do not mix with components from other manufacturers.
7. Do not use reagents beyond the stated expiry date.
8. All reagents must be equilibrated to room temperature (20°C - 25°C) before running the assay. Only take an appropriate amount of reagents at once. Do not put unused reagents back into the vials as reagent contamination may occur.
9. Before opening the AAV2 Control, quickly spin the vial to ensure that all the lyophilized powder has collected at the bottom, and prevent the lyophilized powder from splashing when opening the lid.
10. Use only distilled or deionized water and clean glassware.
11. Do not let wells dry during the test; add reagents immediately after completing washing steps.

VII. SPECIMEN COLLECTION AND STORAGE

1. The handling and storage information provided here is intended to be used as a general guideline. Sample stability has not been evaluated. When samples need to be stored for a long time, users need to evaluate the stability of the samples. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria that meet their needs.
2. Store specimens at -20°C or lower if not testing immediately. Avoid repeated freeze-thaw cycles.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

VIII. PROTOCOL

- **Reagent Preparation**

All reagents must be equilibrated to room temperature before use (20°C to 25°C). All samples and reagents, except AAV2 Control, should be vortexed before use. Store all reagents back in refrigerator promptly after use.

1× Wash Solution: Dilute the 20× Wash Solution with deionized or distilled water with a volume ratio of 1:19. For example, dilute 40 mL of 20× Wash Solution with 760 mL of deionized or distilled water to make 800 mL of 1× Wash Solution. Store the solution at 2°C to 8°C when not in use.

Note: If any precipitate is found in the 20× Wash Solution, incubate the bottle in a water bath (up to 50°C) with occasional mixing until all the precipitate is dissolved.

Standard working solution and sample preparation

This kit provides standards for AAV2 only. It is recommended to use a positive control that matches the AAV capsids in the samples to generate an accurate standard curve for quantification. The user could optimize and validate the range for each specific serotype.

Reconstitute AAV2 Control with 100 µL of deionized or distilled water to make AAV2 standard working solution. Mix AAV2 standard working solution by rolling and incubate for 5 min at RT. Avoid vortex. Find the amount of capsids/mL on the label.

Reconstituted AAV2 Control or other serotype standards prepared and the samples to be tested are processed as follows:

1. Add 50 µL of AAV2 Control, other serotype standards, or AAV unknown samples to individual tubes, respectively.
2. Add 5.6 µL of 10× Lysis Buffer to each tube and mix thoroughly.

Note: If the 10× Lysis Buffer solidifies during storage at 4°C, incubate it at 37°C for 1 minute with occasional mixing until the solution is fully clarified.

3. Incubate each tube at 99±2 °C for 10 minutes.
4. Transfer each tube to room temperature and stand for 5 minutes.
5. Centrifuge briefly each tube for 30 seconds.
6. Label the processed AAV2 Control or other serotype standards as standard working solution. Standard working solution concentration = the concentration of AAV2 Control or other serotype standards × 0.9.
7. Store unused standard working solution or prepared unknown samples at -20°C to avoid repeated freeze-thaw cycles.

Sample dilution

Perform "Sample dilution" after "Standard working solution and sample preparation". Dilute

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

the prepared sample with Sample Dilution Buffer. We recommend at least a 20-fold dilution to avoid interference from Lysis Buffer. Unknown samples, especially samples with very high titer of AAV, must be diluted prior to the assay to obtain an accurate titer value of AAV which is within the linear range of the kit.

It is recommended that all samples be prepared in duplicate. The results are multiplied by the dilution factor to determine the AAV value in the original sample.

Standard working solution dilution

Perform "Standard working solution dilution" after "Standard working solution and sample preparation".

Note: This kit provides standards for AAV2 only. After preparing the "Standard Working Solution and Sample Preparation", construct the AAV2 standard curve using the dilution method below. For other serotypes, operators must prepare their own standards, as dilution methods vary. The detection range for other serotypes is in "XI. ANALYTICAL PERFORMANCE. Assay Working Ranges" for standard curve design.

Dilute AAV2 standard working solution with Sample Dilution Buffer with a volume ratio of 1:30. For example, mix 20 µL of AAV2 standard working solution with 580 µL of Sample Dilution Buffer to make 600 µL of standard solution with label of “Std-01”.

$$\text{Concentration of Std-01} = \frac{\text{Concentration of AAV2 Standard working solution}}{30}$$

We recommend to dilute the standard solution with Sample Dilution Buffer with a volume ratio of 1:2 according to the steps below.

Prepare seven 1.5 mL tubes labeled numerically from Std-02 to Std-08 consecutively, and pipette 250 µL of Sample Dilution Buffer into each tube.

Pipette 250 µL of the standard solution from Std-01 tube into the Std-02 tube and mix. Then pipette 250 µL of the standard solution from the former tube into the latter one according to these steps.

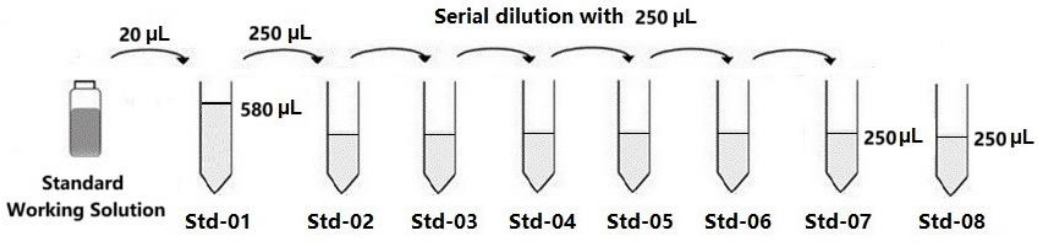

Figure 1. Diagram of Serial Dilution

- **Capture Plate Preparation**

1. It is recommended that all standards be prepared at least in duplicate.
2. Count the strips according to the number of test samples and install the strips. Make sure the strips are tightly snapped into the plate frame.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

3. Leave the unused strips in the foil pouch and store at 2°C to 8°C. The strips must be stored in the closed foil pouch to prevent moisture from damaging the Capture Plate.

Table 1. Setup of standards and samples on Capture Plate

	Standards		Samples									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	Std-1	Std-1	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	Std-2	Std-2	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	Std-3	Std-3	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	Std-4	Std-4	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	Std-5	Std-5	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	Std-6	Std-6	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	Std-7	Std-7	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	Std-8	Std-8	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Std: Standard number； **S:** Sample number

- **Test Procedure**

Standards and Samples Incubation

1. Add 100 µL of the processed AAV2 standards or other serotype standards, and samples to the corresponding wells in the Capture Plate.
2. Cover the plate with Plate Sealer and incubate at 37°C for 60 minutes.
3. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
4. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after the washing steps.

Detection Antibody Incubation

5. Add 100 µL of Detection Antibody to all the testing wells.
6. Cover the plate with Plate Sealer and incubate at 37°C for 30 minutes.
7. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
8. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after washing steps.

Enzyme Conjugate Incubation

9. Add 100 µL of Streptavidin-HRP to all the testing wells.
10. Cover the Plate with Plate Sealer and incubate at 37°C for 10 minutes.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188

11. Remove the Plate Sealer and wash the plate with 260 µL of 1× Wash Solution for four times.
12. Tap inverted plate onto absorbent paper to remove residual liquid in the wells after washing steps.

Substrate Reaction and Absorbance Measurement

13. Add 100 µL of TMB Solution to each well and incubate the plate in dark at 25°C for 15 minutes (start timing after the addition of TMB Solution to the first well).
14. Add 50 µL of Stop Solution to each well to stop the reaction.
15. Read the absorbance in microplate reader at dual wavelengths of 450 nm immediately.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

IX. ASSAY PROCEDURE SUMMARY

1. • Processe the AAV2 Control or other serotype standards, as well as the samples, with 10× Lysis Buffer at 99±2 °C for 10 minutes.
2. • Dilute the test samples and a set of standards with Sample Dilution Buffer.
3. • Add 100 µL of the diluted samples/standards to the corresponding wells. Incubate the plate at 37°C for 60 minutes.
4. • Wash the plate with 260 µL of 1× Wash Solution per well for four times.
5. • Add 100 µL of the Detection Antibody to the well and incubate at 37°C for 30 minutes.
6. • Wash the plate with 260 µL of 1× Wash Solution per well for four times.
7. • Add 100 µL of the Streptavidin-HRP and incubate at 37°C for 10 minutes.
8. • Wash the plate with 260 µL of 1× Wash Solution per well for four times.
9. • Add 100 µL of TMB Solution and incubate the plate in dark at 25°C for 15 minutes.
10. • Add 50 µL of Stop Solution to each well to stop the reaction.
11. • Read the plate immediately.

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

X. INTERPRETATION OF RESULTS

- **Assay Validation**

To ensure the validity of results, the following criteria noted in the Table 2 are required. If a test fails to meet the requirements, the test is invalid and must be repeated.

Table 2. OD_{450} values for quality control

Items	Special Test	Requirements
1	OD_{450} value for Std-01	> 1.2
2	OD_{450} value for Std-08	< 0.2

Note: The standards in the table are only intended to evaluate the performance of the kit.

- **Suggested Calculation of Data**

Statistical software can be used to create the standard curves. Choose a method with high Goodness of Fit (R^2) to analyze the data, such as linear regression or a four-parameter logistic (4-PL) model that provides point-to-point curve fitting.

The standard curve is for demonstration purposes only. It should be prepared each time an assay is performed. The standard curve should be calculated based on the concentration specified on the label of the standard.

Table 3. Typical AAV2 standard curve data with a reconstituted AAV2 Control concentration of 2.33E11 capsids/mL.

Standard	OD Value (450 nm)		
(Capsids/mL)	Duplicate 1	Duplicate 2	Average
7.00E+09	2.1149	2.0493	2.0821
3.50E+09	1.0456	0.9871	1.0164
1.75E+09	0.5526	0.5259	0.5393
8.75E+08	0.3066	0.3035	0.3051
4.38E+08	0.1864	0.1789	0.1827
2.19E+08	0.1232	0.1190	0.1211
1.09E+08	0.1049	0.0939	0.0994
0	0.0783	0.0734	0.0759

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

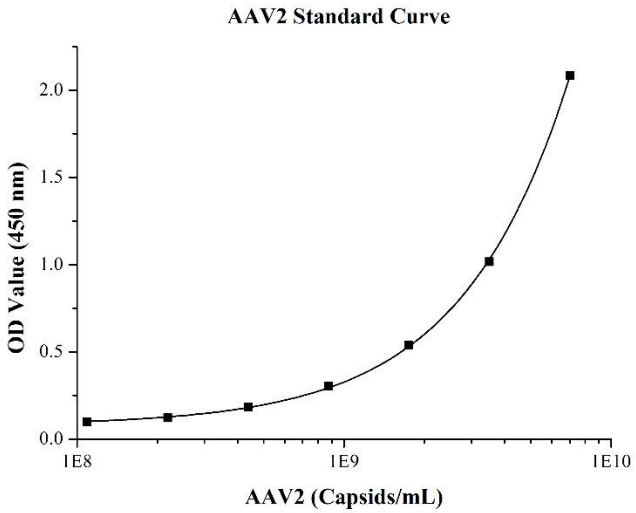

Figure 2. A typical example of AAV2 standard curve using a four-parameter logistic curve. Each standard was analyzed in duplicate. The R-Square of this curve is 0.99977.

XI. ANALYTICAL PERFORMANCE

- **Assay Working Ranges**

The typical detection range of this kit for AAV2 is from 7.83E+07 to 7.0E+09 capsids/mL. The antibodies in this kit's raw material recognize AAV of different serotypes differently, so the typical assay working range of AAV varies when testing different serotypes, as shown in Table 4 and Figure 3.

The antibody in this kit also recognizes AAVDJ and AAVrh.1, but this kit is not validated for the detection of these two AAVs. Although the remaining serotypes of AAV are not validated in this kit, it does not necessarily mean that they cannot be detected. Users can follow the protocol of this kit to evaluate the suitability of the test for other serotypes.

Table 4. Typical assay working ranges for different AAV serotypes.

Serotype	Information and Sources of Reference Standards	Typical assay working ranges (Capsids/mL)
AAV2	AAV2 VLP (GenScript)	1.32E+08 - 7.00E+09
AAV1	AAV1 empty capsids (Progen, 66V010)	2.73E+07 - 1.75E+09
AAV5	AAV5 empty capsids (Progen, 66V050)	6.30E+07 - 4.00E+09
AAV6	AAV6 empty capsids (Progen, 66V060)	3.91E+07 - 2.50E+09
AAV8	AAV8 empty capsids (Progen, 66V080)	6.27E+07 - 4.00E+09
AAV9	AAV9 empty capsids (Progen, 66V090)	1.09E+08 - 7.00E+09

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

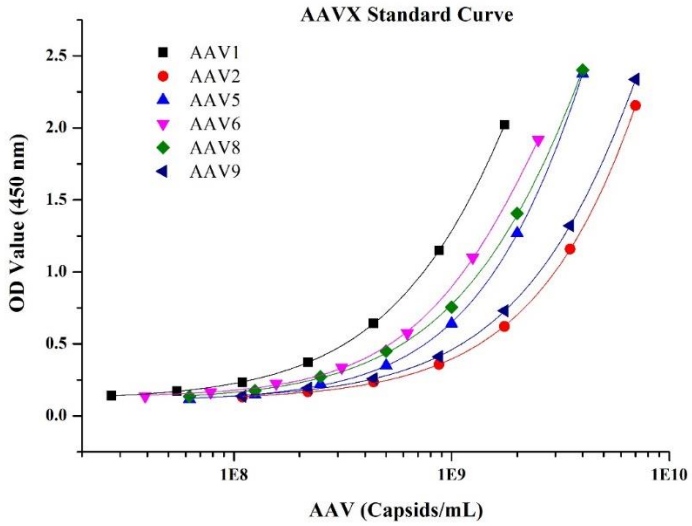

Figure 3. Overlay plot of standard curves for different AAV serotypes.

- **Detection Capability**

  According to the evaluation method recommended in CLSI guideline EP17-A2[1], the LoB (Limit of Blank) of this kit is 3.50E+07 Capsids/mL.

  According to the evaluation method recommended in CLSI guideline EP17-A2[1], the LoD (Limit of Detection) for AAV2 detection is 7.83E+07 Capsids/mL and the LoQ (Limit of Quantitation) for AAV2 detection is 1.16E+08 Capsids/mL.

- **Measurement Precision**

  Intra-assay and inter-assay precision were measured in 3 pools of different concentrations, using 3 lots of kits.

Table 5. Intra-assay CV evaluation with AAVX Titer Capsid ELISA Kit - Multiple Serotypes

Batch No.	Repeats	Average Measured Conc. (Capsids/mL)	% CV	Theoretical Conc. (Capsids/mL)	Recovery Rate (%)
#1	10	4.03E+08	**3.58**	4.00E+08	101
	10	1.24E+09	**3.15**	1.20E+09	103
	10	4.70E+09	**1.80**	5.00E+09	94
#2	10	4.32E+08	**4.07**	4.00E+08	108
	10	1.28E+09	**5.77**	1.20E+09	107
	10	4.58E+09	**6.30**	5.00E+09	92
#3	10	3.94E+08	**5.93**	4.00E+08	99
	10	1.24E+09	**8.21**	1.20E+09	103
	10	4.50E+09	**5.81**	5.00E+09	90

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

Table 6. Inter-assay CV evaluation with three-batch AAVX Titer Capsid ELISA Kit - Multiple Serotypes

Batch Amount	Repeats	Average Measured Conc. (Capsids/mL)	% CV	Theoretical Conc. (Capsids/mL)	Recovery Rate (%)
3	3×10	4.09E+08	**5.93**	4.00E+08	102
3	3×10	1.26E+09	**5.95**	1.20E+09	105
3	3×10	4.59E+09	**5.07**	5.00E+09	92

- **Spike & Recovery**

  This experiment means spiking a known amount of standard to the buffer matrix and then testing whether the spiked standard can be recovered quantitatively. Extreme pH, high salt concentrations, some high protein concentrations, and some detergents may result in inadequate recovery. The user can dilute the standards provided in this kit into the sample matrix of interest and perform recovery experiments to identify whether the sample can be accurately recovered in the matrix. This is necessary to determine the accuracy of the experiment.

Table 7. Recovery rate of AAVX Titer Capsid ELISA Kit - Multiple Serotypes in triplicates

Spiked Conc. (Capsids/mL)	Batch No.	Recovery Rate
1.50E+08	#1	98%
	#2	109%
	#3	94%

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854
1-732-885-9188

XII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Weak/No Signal	Substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	Substrate is contaminated	Use new Substrate from the same Lot
	Volumes of reagents are not correct	Repeat assay with the required volumes as noted in the manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat the assay
	The plate is not read within the specified time range	Read the plate within 5 minutes
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	Substrate is contaminated	Use new substrate from the same Lot
	Evaporation of wells during incubations	Perform incubation steps with a plate sealer in a repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay

XIII. REFERENCES

1. CLSI document EP17-A2 (Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition).

For research use only. Not intended for human or animal clinical trials, therapeutic or diagnostic use.

Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188