

AAV total capsid ELISA with CaptureSelect™ anti-AAV affinity reagents

Utilizing broad serotype coverage and high affinity of the CaptureSelect AAVX affinity ligand for quantification of AAV total capsids in a Sandwich ELISA set-up

 The world leader in serving science



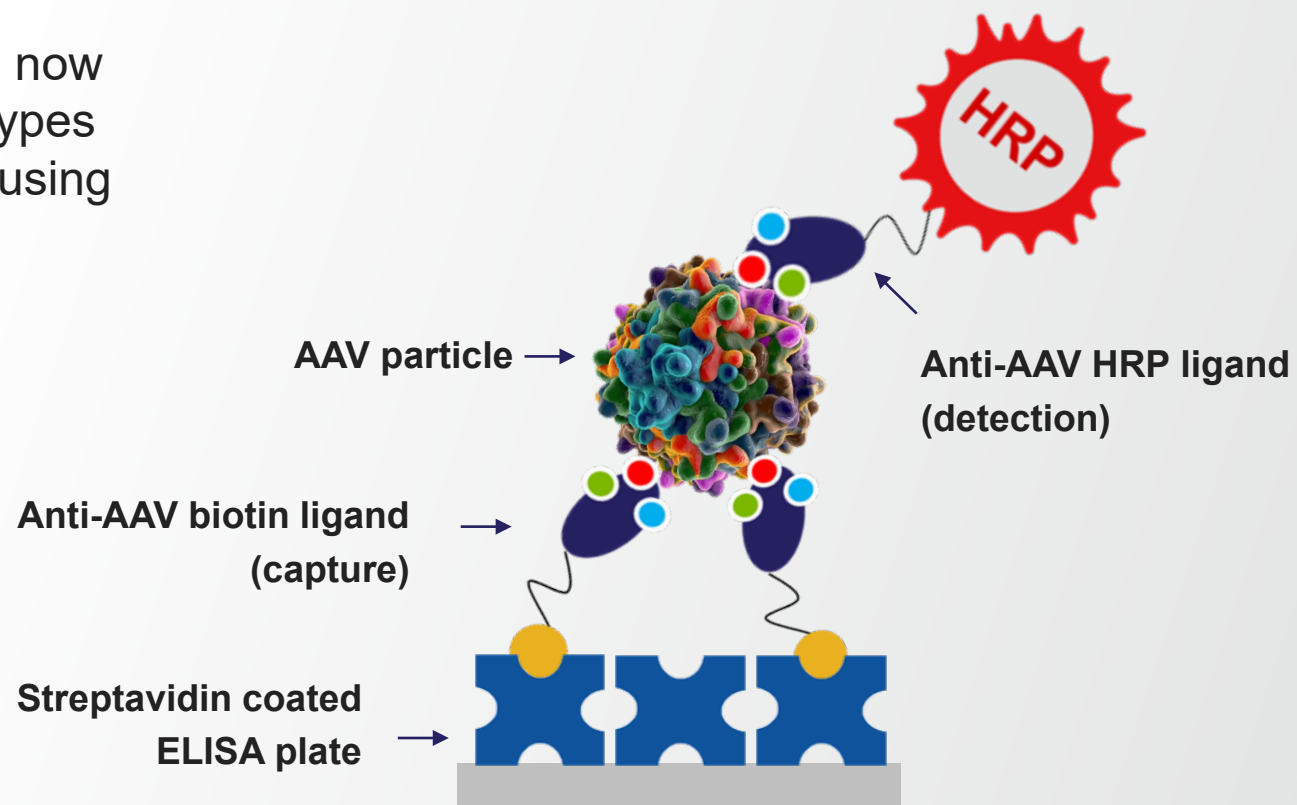
Total AAV capsid ELISA using CaptureSelect™ anti-AAV affinity reagents

POROS™ CaptureSelect™ AAV affinity resins are specifically developed to improve the downstream purification of Adeno-Associated Virus (AAV). The POROS™ CaptureSelect™ AAVX resin allows purification of a broad range of naturally occurring and synthetic AAV serotypes used in gene therapy applications, making it an ideal platform for AAV manufacturing.

The benefits provided by the AAVX affinity ligand can now be utilized for the quantification of multiple AAV serotypes in cell culture supernatants or purified AAV samples, using the sandwich ELISA technology

Total AAV capsid ELISA using CaptureSelect anti-AAV affinity reagents:

- CaptureSelect Biotin AAVX or AAV9 ligands are used as capture reagents
- CaptureSelect AAVX or AAV9 HRP ligands are used as detection reagents



CaptureSelect anti-AAV affinity reagents

Capture Antibody – Biotin conjugate	Cat.No.	Unit Size (µg)	Working range (µg/ml)	No. of ELISA plates (96 wells)
CaptureSelect™ Biotin Anti-AAVX Conjugate <i>Broad AAV serotypes coverage, including AAV1 to AAV8, and AAVrh10.</i>	7103522100	100	2 - 0.25	5 – 40
	7103522500	500		25 – 200
CaptureSelect™ Biotin Anti-AAV9 Conjugate <i>AAV9 serotype specific</i>	7103332100	100	1 - 0.05	10 – 100
	7103332500	500		50 – 500

Buffer: PBS,

Storage: in freezer (-5 to -30°C)

Detection Antibody – HRP conjugate	Cat.No.	Unit Size (µl)	Working range (dilution)	No. of ELISA plates (96 wells)
CaptureSelect™ HRP Anti-AAVX Conjugate	7303522100	100	1:5000 – 1:20000	50 – 200
CaptureSelect™ HRP Anti-AAV9 Conjugate	7303332100	100	1:5000 – 1:20000	50 – 200

Buffer: PBS, 50% (v/v) glycerol

Storage: in freezer (-5 to -30°C)

AAV Total Capsid ELISA - *recommended starting protocol*

1

Coat plate with AAV capture ligand

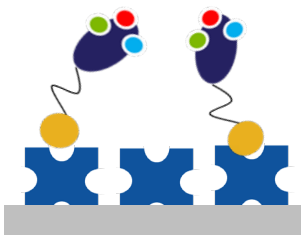
Incubate Streptavidin coated plates with biotinylated anti-AAV affinity ligand

Streptavidin coated plates

Pierce™ Streptavidin Pre-Coated Plates.

Antibody Coating range:

- Biotin anti-AAVX: 0.25 – 2 µg/ml
- Biotin anti-AAV9: 0.05 – 1 µg/ml



1 hr

WASH*

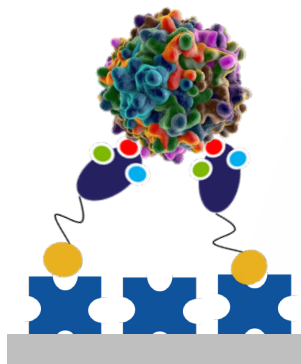
2

Incubation with AAV sample

Incubate antibody coated plates with AAV sample

Concentration range:

for standard curves
• $\sim 10^8 - 10^{11}$ AAV particles / ml



1 hr

WASH*

3

Incubation with AAV detection ligand

Incubate with HRP labeled anti-AAV affinity ligand

Working dilution range:

- HRP anti-AAVX: 1:5000 – 1:20000
- HRP anti-AAV9: 1:5000 – 1:20000



1 hr

WASH*

4

Detection

Visualize binding through substrate color reaction

Reagents and measurement:

- 1-Step™ Ultra TMB-ELISA Substrate
- Stop solution: 1M H₂SO₄
- Measure: Absorbance (450nm)



5-10 min

*Wash: Wash plates 3X with PBS 0.05% tween-20

AAV1 total capsid ELISA using AAVX ligands (example)

Streptavidin Pre-Coated plate

1

Biotin anti-AAVX conjugate at
1 µg/ml
in PBST*, 100 ul/well, 1 hr, RT

2

AAV1 capsids titration
 1×10^8 - 3×10^{10} particles/ml
in PBST*, 100 ul/well, 1 hr, RT

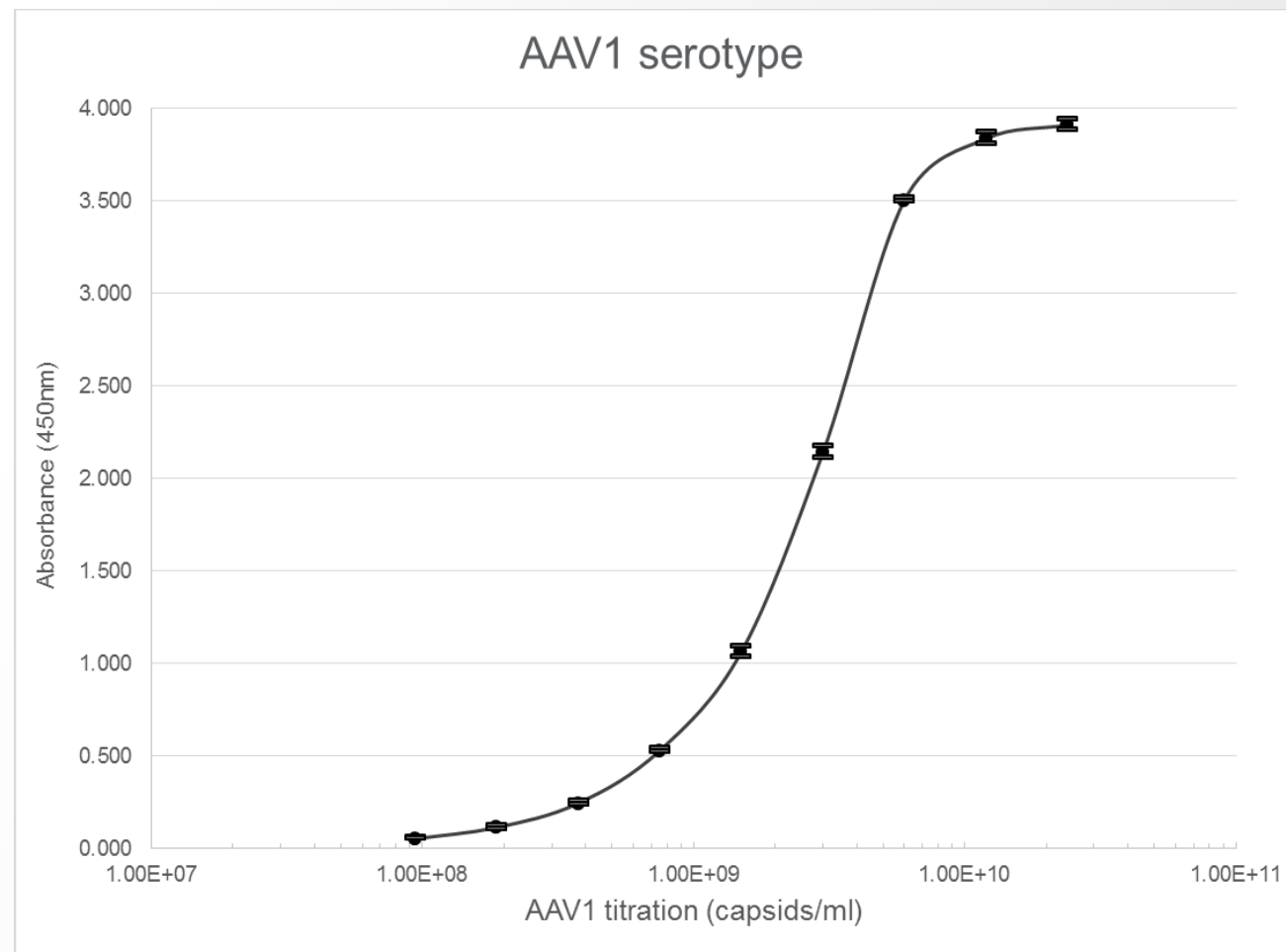
3

HRP anti-AAVX conjugate dilution
1:10,000
in PBST*, 100 ul/well, 1 hr, RT

4

Ultra TMB ELISA substrate
10 minutes
100 ul/well, RT

Stop with 1M H₂SO₄, 50 µl/well
Measure absorbance at 450 nm



AAV4 total capsid ELISA using AAVX ligands (example)

Streptavidin Pre-Coated plate

1

Biotin anti-AAVX conjugate at
1 µg/ml
in PBST*, 100 ul/well, 1 hr, RT

2

AAV4 capsids titration
 2×10^8 - 8×10^{10} particles/ml
in PBST*, 100 ul/well, 1 hr, RT

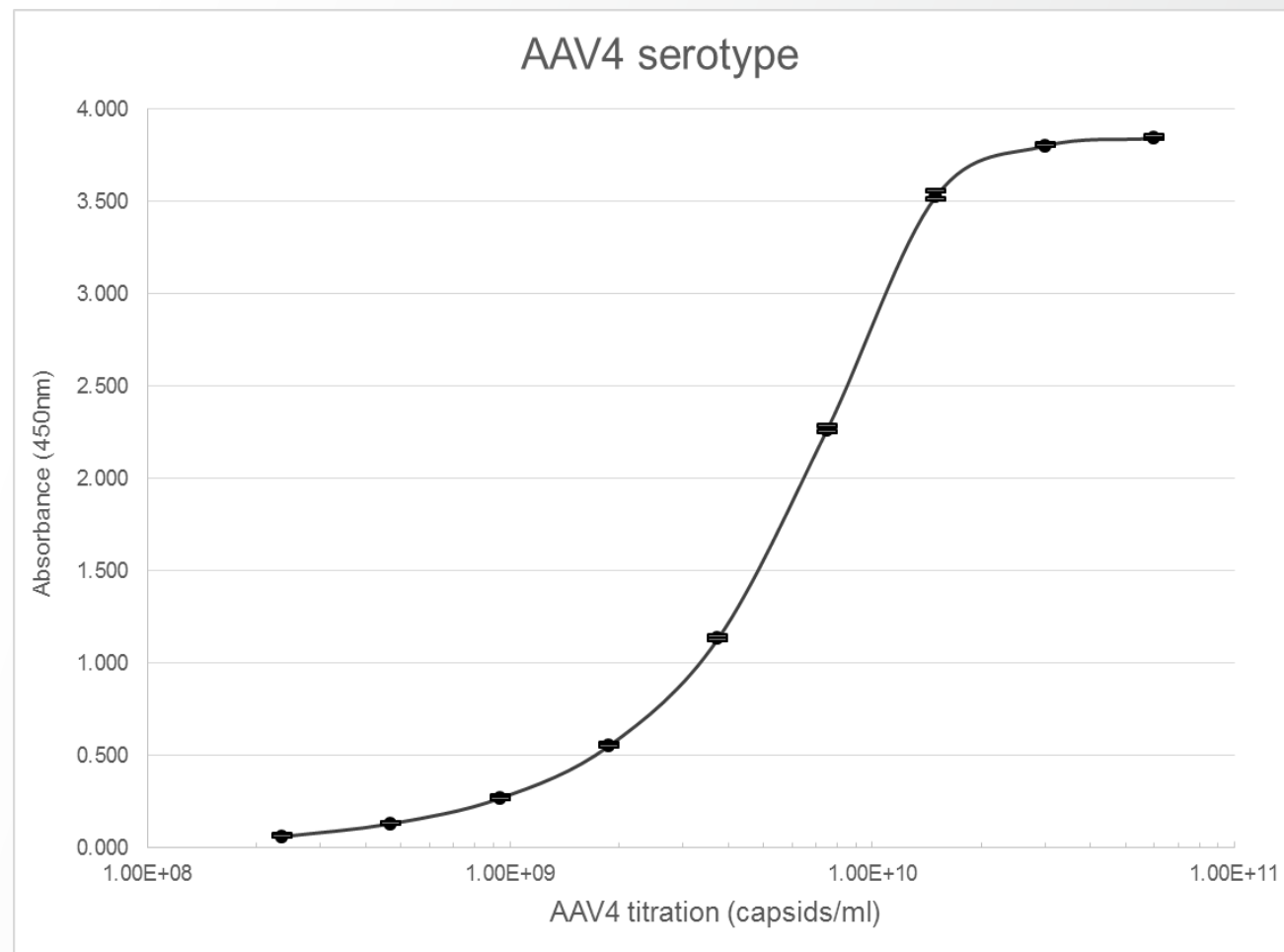
3

HRP anti-AAVX conjugate dilution
1:10,000
in PBST*, 100 ul/well, 1 hr, RT

4

Ultra TMB ELISA substrate
10 minutes
100 ul/well, RT

Stop with 1M H₂SO₄, 50 µl/well
Measure absorbance at 450 nm



AAV9 total capsid ELISA using AAV9 ligands (example)

Streptavidin Pre-Coated plate

1

Biotin anti-AAV9 conjugate at
0.2 µg/ml
in PBST*, 100 ul/well, 1 hr, RT

2

Purified AAV9 capsids titration
 8×10^7 - 1×10^{10} particles/ml
in PBST*, 100 ul/well, 1 hr, RT

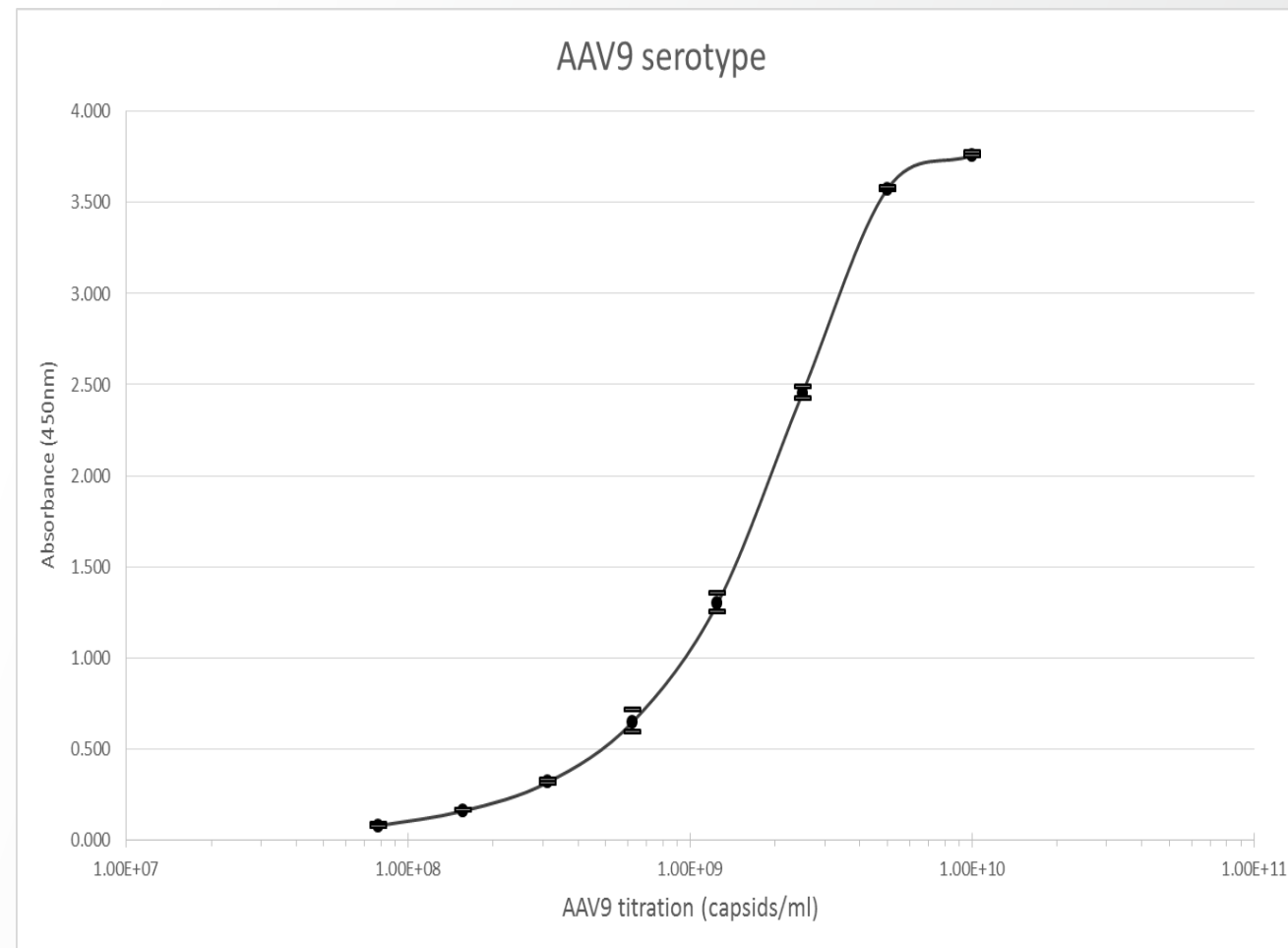
3

HRP anti-AAV9 conjugate dilution
1:10,000
in PBST*, 100 ul/well, 1 hr, RT

4

Ultra TMB ELISA substrate
5 minutes
100 ul/well, RT

Stop with 1M H₂SO₄, 50 µl/well
Measure absorbance at 450 nm



Additional remarks

- The assay described in this guide is tested in house, but not validated, and is intended as a starting point for protocol development and optimization. In addition, further testing and optimization of assay conditions is needed for each AAV serotype.
 - *Biotin anti-AAV conjugate concentration for coating on streptavidin functionalized plates*
 - *AAV concentration range*
 - *HRP anti-AAV conjugate dilution*
 - *color time*

Standard curve creation

- Thermo Fisher Scientific does not supply standards for standard curve development. It is recommended to use purified AAV capsids, which most resemble the AAV vector of interest, as a standard.
- When using CaptureSelect AAV biotinylated and HRP conjugated affinity ligands, the following AAV capsid concentration ranges can be used as a starting point for standard curve creation:

CaptureSelect AAVX Sandwich ELISA:

- *AAV1 to AAV7 and AAVrh10* $1 \times 10^8 - 1 \times 10^{11}$ capsids / ml
- *AAV8* $1 \times 10^9 - 1 \times 10^{12}$ capsids / ml
- *AAV9 not suitable with AAVX reagents*

CaptureSelect AAV9 Sandwich ELISA:

- *AAV9* $5 \times 10^7 - 5 \times 10^{11}$ capsids / ml

AAV sandwich ELISA – ordering table

Product	Product nr & link to website
Pierce™ Streptavidin Coated plates, Clear, 96-Well <small>(Clear, 96-well plates, coated with 100uL of Streptavidin and blocked with 200uL of SuperBlock Blocking Buffer)</small>	15124 (5 plates) 15126 (5x 5 plates)
1-Step™ Ultra TMB ELISA substrate solution	34028 (250mL) 34029 (1L)
Pierce™ 20x PBS Tween™ 20 buffer (PBST) <small>(diluted 20-fold in water, the solution yields 10 mM sodium phosphate, 0.15M NaCl, 0.05% Tween-20, pH 7.5)</small>	28352 (500 mL)
CaptureSelect™ Biotin Anti-AAVX Conjugate (Capture ligand)	7103522100 (100 µg) 7103522500 (500 µg)
CaptureSelect™ HRP Anti-AAVX Conjugate (Detection ligand)	7303522100 (100 µl)
CaptureSelect™ Biotin Anti-AAV9 Conjugate (Capture ligand)	7103332100 (100 µg) 7103332500 (500 µg)
CaptureSelect™ HRP Anti-AAV9 Conjugate (Detection ligand)	7303332100 (100 µl)