Physical particle titer assay using Progen AAV8 Titration ELISA

Use the Progen protocol that is provided with the kit for the titration (20-390 PRAAV8).
Dilute the reference standard with ready-to-use sample buffer so that you can measure within the linear range of the ELISA (1.47x 10E7- 9.24 x 10E8 capsids/ml)
The assay will measure empty and full capsids.

For each of the two replicate assays prepare the following volumes of 1X solution from 20X stock solutions supplied in the kit:

Ready to use sample buffer: 10mL
Wash buffer: 100mL
Anti-AAV8 biotin conjugate (20X is reconstituted in 750ul dH_2O): 6mL
Stepavidin peroxidise conjugate: 6mL

Reconstitute Kit standard (STD) in 500ul dH_2O.
Dilute Kit standard in ready-to-use sample buffer in steps of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 in Eppendorf vials.
1:2 250 µl ready-to-use sample buffer + 250 µl reconstituted Kit Control, vortex

1:4 250 µl ready-to-use sample buffer + 250 µl from 1:2 diluted Kit Control, vortex
etc.

Dilute reference standard in ready-to use sample buffer:
990 µl sample buffer + 10 µl reference standard (=1:100 predilution), vortex.

From this dilution make 1:1000, 1:2000, 1:4000, 1:8000, 1:16000 dilutions in Eppendorf vials.

1:1000	450 µl ready-to-use sample buffer + 50 µl of 1:100 prediluted reference standard, vortex
1:2000	250 µl ready-to-use sample buffer + 250 µl of 1:1000 dilution of reference standard, vortex
1:4000	250 µl ready-to-use sample buffer + 250 µl of 1:2000 dilution of reference standard, vortex
1:8000	250 µl ready-to-use sample buffer + 250 µl of 1:4000 dilution of reference standard, vortex
1:16000	250 µl ready-to-use sample buffer + 250 µl of 1:8000 dilution of reference standard, vortex

These dilutions are made 4 times independantly from the 1:100 prediluted standard.
1 st dilution 1:2000 – 1:16000
2 nd dilution 1:2000 – 1:16000
3 rd dilution 1:2000 – 1:16000
4 rth dilution 1:2000 – 1:16000

All samples are applied in duplicate on the microtiter plate.

Application of samples on the Microtiter plate:

	1	2	3	4	5	6	7	8	9	10	11	12
A	KC undiluted	KC undiluted	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000						
B	KC 1:2	KC 1:2	Ref.std 1:4000	Ref.std 1:4000	Ref.std 1:4000	Ref.std 1:4000						
C	KC 1:4	KC 1:4	Ref.std 1:8000	Ref.std 1:8000	Ref.std 1:8000	Ref.std 1:8000						
D	KC 1:8	KC 1:8	Ref.std 1:16000	Ref.std 1:16000	Ref.std 1:16000	Ref.std 1:16000						
E	KC 1:16	KC 1:16	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000						
F	KC 1:32	KC 1:32	Ref.std 1:4000	Ref.std 1:4000	Ref.std 1:4000	Ref.std 1:4000						
G	KC 1:64	KC 1:64	Ref.std 1:8000	Ref.std 1:8000	Ref.std 1:8000	Ref.std 1:8000						
H	Sample buffer	Sample buffer	Ref.std 1:16000	Ref.std 1:16000	Ref.std 1:16000	Ref.std 1:16000						

KC = Kit Control
Ref.std = reference standard

Proceed with the assay as described on the kit instuctions.

Repeat the assay on another day with the second half of the microtiter plate. If available use software with 4-parameter fit to plot the standard curve and deduce the concentration of the reference standard form that curve.