Physical particle titer assay using Progen AAV2 Titration ELISA

Use protocol that is provided with the kit for the titration.
Dilute the reference standard with ready-to-use sample buffer so that you can measure within the linear range of the ELISA (5 x 10E7-5 x 10E9capsids/ml)
The assay will measure empty and full capsids.

Reconstitute Kit Control according to protocol included in test kit.
Dilute Kit Control 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:
490 µl sample buffer + 10 µl reference standard (=1:50 predilution), vortex.

From this dilution make 1:250, 1:500, 1:1000 and 1:2000 dilutions in Eppendorf vials.

1:250	400 µl ready-to-use sample buffer + 100 µl of 1:50 prediluted reference standard, vortex
1:500	250 µl ready-to-use sample buffer + 250 µl of 1:250 dilution of reference standard, vortex
1:1000	250 µl ready-to-use sample buffer + 250 µl of 1:500 dilution of reference standard, vortex
1:2000	250 µl ready-to-use sample buffer + 250 µl of 1:1000 dilution of reference standard, vortex

These dilutions are made 4 times independantly from the 1:50 prediluted standard.
1 st dilution 1:250 – 1:2000
2 nd dilution 1:250 – 1:2000
3 rd dilution 1:250 – 1:2000
4 rth dilution 1:250 – 1:2000

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 1:250	Ref.std 1:250	Ref.std 1:250	Ref.std 1:250						
B	KC 1:2	KC 1:2	Ref.std 1:500	Ref.std 1:500	Ref.std 1:500	Ref.std 1:500						
C	KC 1:4	KC 1:4	Ref.std 1:1000	Ref.std 1:1000	Ref.std 1:1000	Ref.std 1:1000						
D	KC 1:16	KC 1:16	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000						
E	KC 1:32	KC 1:32	Ref.std 1:250	Ref.std 1:250	Ref.std 1:250	Ref.std 1:250						
F	KC 1:64	KC 1:64	Ref.std 1:500	Ref.std 1:500	Ref.std 1:500	Ref.std 1:500						
G	KC 1:128	KC 1:128	Ref.std 1:1000	Ref.std 1:1000	Ref.std 1:1000	Ref.std 1:1000						
H	Sample buffer	Sample buffer	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000	Ref.std 1:2000						

KC = Kit Control
Ref.std = reference standard

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.