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 10E9 capsids/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 independently from the 1:50 prediluted standard.
1st dilution 1:250 – 1:2000
2nd dilution 1:250 – 1:2000
3rd dilution 1:250 – 1:2000
4th dilution 1:250 – 1:2000

All samples are applied in duplicate on the microtiter plate.
Application of samples on the Microtiter plate:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>KC undiluted</td>
<td>KC undiluted</td>
<td>Ref.std 1:250</td>
<td>Ref.std 1:250</td>
<td>Ref.std 1:250</td>
<td>Ref.std 1:250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>KC 1:2</td>
<td>KC 1:2</td>
<td>Ref.std 1:500</td>
<td>Ref.std 1:500</td>
<td>Ref.std 1:500</td>
<td>Ref.std 1:500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>KC 1:4</td>
<td>KC 1:4</td>
<td>Ref.std 1:1000</td>
<td>Ref.std 1:1000</td>
<td>Ref.std 1:1000</td>
<td>Ref.std 1:1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>KC 1:32</td>
<td>KC 1:32</td>
<td>Ref.std 1:250</td>
<td>Ref.std 1:250</td>
<td>Ref.std 1:250</td>
<td>Ref.std 1:250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>KC 1:64</td>
<td>KC 1:64</td>
<td>Ref.std 1:500</td>
<td>Ref.std 1:500</td>
<td>Ref.std 1:500</td>
<td>Ref.std 1:500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>KC 1:128</td>
<td>KC 1:128</td>
<td>Ref.std 1:1000</td>
<td>Ref.std 1:1000</td>
<td>Ref.std 1:1000</td>
<td>Ref.std 1:1000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.