**Description**

AAV8 Chemiluminescence ELISA Titration Kit is a sandwich ELISA (Enzyme-linked immunosorbent assay) designed to detect and quantify AAV8 (adeno-associated virus type 8) non-denatured capsids. It provides accurate, specific, and sensitive assessment of the number of AAV8 capsids (full and empty) present in purified AAV8 samples. This kit contains all the materials necessary for 100 reactions, including high sensitivity ELISA ECL reagents. This kit has a linear range between $7.8x10^7$ - $1x10^{10}$ capsids/ml.

*Note: The capture and detection antibodies detect a conformational epitope not present on unassembled capsid proteins.*

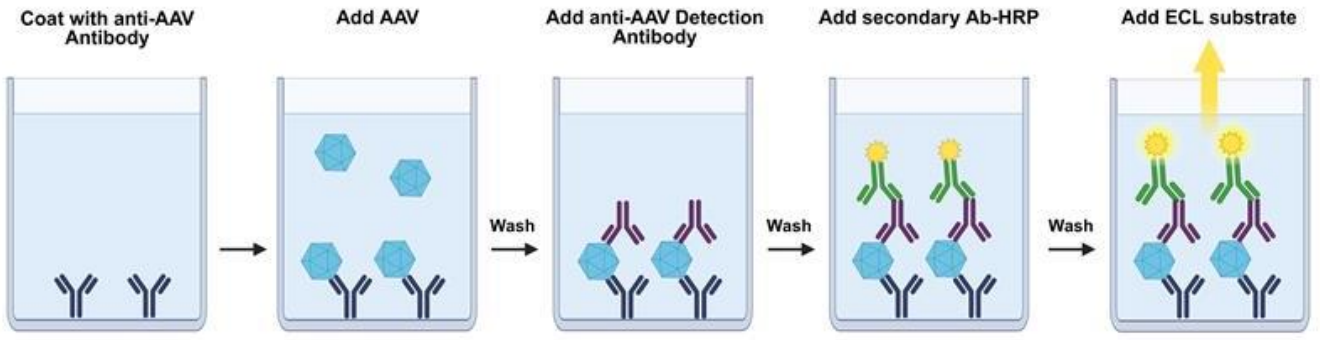


*Figure 1. AAV8 Chemiluminescence ELISA Titration Kit schematic.*
Coat the plate with the Anti-AAV8 Coating Antibody. Prepare a serial dilution of the reference virion and of your samples of interest, add to the coated plate, wash, and add the Anti-AAV8 Detection Antibody followed by anti-HRP-labeled secondary antibody. The chemiluminescence signal is directly correlated with the number of AAV8 capsids present.

**Background**

Recombinant adeno-associated virus (rAAV) is a widely used vector in gene therapy applications. rAAVs exist in the nucleus as episomes and are unable to integrate into the cellular genome. While this brings safety benefits, as there is no risk of random integration and activation of oncogenes, the cell division rate dictates the half-life for transgene expression. Their application is thus particularly useful for the delivery of transgenes to post-mitotic tissues like the brain, retinal, liver, skeletal muscle, and the heart. The payload carried by AAVs is somewhat smaller than other viral tools used for transduction, usually smaller than 5 kb. There are several serotypes, each one with a specific tissue tropism. During production, AAV titers can be measured as a physical titer, infectious titer, or ratio of full to empty capsids. Accurately determining the titer is essential not only for setting clinical doses but also as a foundational step in various analytical assays for characterizing AAV products. The use of an ELISA-based assay to measure AAV8 particles allows for better inter- and intra-laboratory consistency, compared to the use of primers in qPCR. This is a well-suited kit for adoption in gene therapy research and can be further automated for high-throughput applications.

**Applications**

- Measuring titer of AAV8 samples.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 83796-KC5 | AAV8 Reference | 5 µl | -80°C |
| 83797-KC10 | Anti-AAV8 Coating Antibody | 10 µg | -80°C |
| 87798-KC1 | Anti-AAV8 Detection Antibody | 1 µg | -80°C |
| 82620-KC5 | 5x PP-02 Buffer | 5 ml | -20°C |
| 79728 | Blocking Buffer 2 | 50 ml | +4°C |
| 52131H | Secondary HRP-Labeled Antibody 2 | 10 µl | -80°C |
| 79670-KC6 | ELISA ECL Substrate A (translucent bottle) | 6 ml | Room Temp |
| | ELISA ECL Substrate B (brown bottle) | 6 ml | Room Temp |
| 83606-KC1 | High Sensitivity ELISA ECL Substrate A | 1 ml | Room Temp |
| | High Sensitivity ELISA ECL Substrate B | 1 ml | Room Temp |
| 79699 | White 96-well plate | 1 | Room Temp |
| | Plate sealer | 2 | Room Temp |

**Materials Required but Not Supplied**

- PBS (Phosphate Buffer Saline)
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- 37°C incubator

**Storage Conditions**

This assay kit will perform optimally for up to **6 months** from the date of receipt when the materials are stored as directed.

**Safety**

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

**Contraindications**

This assay kit is compatible with up to 1% final DMSO concentration.

**Assay Protocol**

- All samples and controls should be performed in duplicate.

- We recommend using AAV8 Reference as “Reference Control” and generating a standard curve for each experiment.
- Detection of shuffled/mutated AAV capsids will depend on the impact of the mutation and it is not guaranteed. We recommend using a suitable shuffled/mutated control as standard.
- The assay should include “Blank”, “Reference Control”, and “Test AAV8” conditions.
- Variation in sample collection, processing and storage may cause differences in sample values.

**Step 1: Coating**

1. Thaw **Anti-AAV8 Coating Antibody** on ice. Briefly spin the tube containing the antibody to recover its full content.
2. Dilute Anti-AAV8 Coating Antibody to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted Anti-AAV8 Coating Antibody to every well.
4. Incubate at 4°C overnight.
5. Wash the plate three times using 200 µl of PBST Buffer per well.
6. Tap the plate onto clean paper towels to remove the liquid.
7. Block the wells by adding 200 µl of **Blocking Buffer 2** to every well.
8. Incubate at Room Temperature (RT) for at least 60 minutes.
9. Wash the plate three times using 200 µl of PBST Buffer per well.
10. Tap the plate onto clean paper towels to remove the liquid.

**Step 2: Reaction**

1. Prepare **1x Assay Buffer** by diluting **5x PP-02 Assay Buffe**r 5-fold with distilled water.
2. Thaw **AAV8 Reference** sample on ice.
3. Prepare a serial dilution of the AAV8 Reference (50 µl/well), starting at $1x10^{10}$ capsids/ml, as described in the table below:

| Dilution Series | Volume of AAV Reference stock or previous dilution (µl) | Volume of 1x Assay Buffer (µl) | capsids/ml |
|---|---|---|---|
| Dilution 1 | Check initial concentration of the AAV8 reference lot# | up to 250 µl | $1 \times 10^{10}$ |
| Dilution 2 | 125 µl of Dilution 1 | 125 µl | $5 \times 10^{9}$ |
| Dilution 3 | 125 µl of Dilution 2 | 125 µl | $2.5 \times 10^{9}$ |
| Dilution 4 | 125 µl of Dilution 3 | 125 µl | $1.25 \times 10^{9}$ |
| Dilution 5 | 125 µl of Dilution 4 | 125 µl | $6.25 \times 10^{8}$ |
| Dilution 6 | 125 µl of Dilution 5 | 125 µl | $3.1 \times 10^{8}$ |
| Dilution 7 | 125 µl of Dilution 6 | 125 µl | $1.6 \times 10^{8}$ |
| Dilution 8 | 125 µl of Dilution 7 | 125 µl | $7.8 \times 10^{7}$ |

4. Add 50 µl of serially diluted AAV8 Reference to the "Reference Control" wells.
5. Prepare AAV8 test sample accordingly.
6. Add 50 µl of AAV8 test sample to the "AAV8 Test" wells.
7. Add 50 µl of 1x Assay Buffer to "Blank" wells.
8. Seal the plate using the plate sealer and incubate at 37°C for 1 hour.
9. Remove plate sealer and discard the liquid.
10. Wash the plate 3 times using 200 µl of PBST per well.
11. Tap the plate onto a clean paper towel to remove the liquid.

**Step 3: Detection**

1. Thaw **Anti-AAV8 Detection Antibody** on ice.
2. Dilute **Anti-AAV8 Detection Antibody** to 0.2 ng/µl with **1x Assay Buffer** (50 µl/well).
3. Add 50 µl of **diluted Anti-AAV8 Detection Antibody** to all wells.
4. Seal the plate using second plate sealer and incubate at 37°C for 1 hour.
5. Remove plate sealer and discard the liquid.
6. Wash the plate 3 times using 200 µl of PBST per well.

7. Tap the plate onto a clean paper towel to remove the liquid.

8. Dilute 1000-fold the **Secondary HRP-Labeled Antibody 2** with Blocking Buffer 2 (50 µl/well).

9. Add 50 µl of diluted Secondary HRP-Labeled Antibody **2** to every well.

10. Incubate at Room Temperature (RT) for 1 hour.

11. Wash the plate 3 times using 200 µl of PBST per well.

12. Tap the plate onto a clean paper towel to remove the liquid.

13. Block the wells by adding 200 µl of Blocking Buffer 2 to every well.

14. Incubate at RT for 10 minutes.

15. Wash the plate 3 times using 200 µl of PBST per well.

16. Tap the plate onto clean paper towels to remove the liquid.

17. Just before use, mix 1 volume of **ELISA ECL Substrate A** and 1 volume of **ELISA ECL Substrate B** (100 µl of mix/ well).

17. Add 100 µl of mix to every well.

18. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

19. If a higher signal is needed, mix 1 volume of High-Sensitivity ELISA ECL Substrate A and 1 volume of High-Sensitivity ELISA ECL Substrate B (10-20 µl of mix/well).

20. Add 10-20 µl of High sensitivity mixture on top of ELISA ECL mixture and read again.

21. The “Blank” value should be subtracted from all other values.

**Reading Chemiluminescence**

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel;

Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of controls.

**Example Results.**

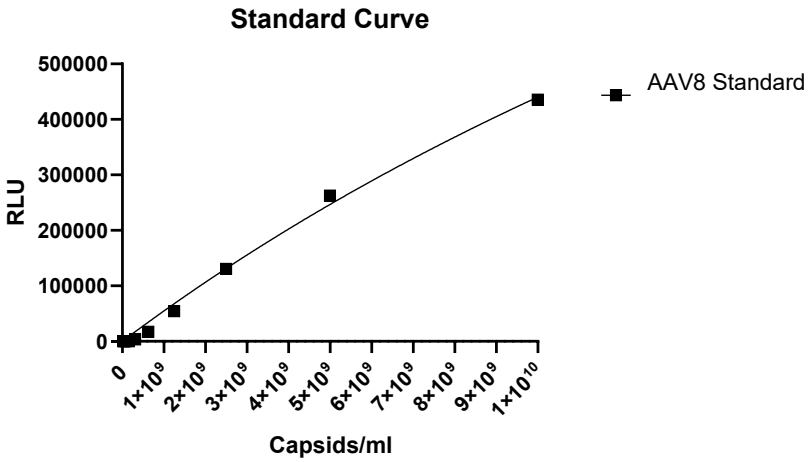


*Figure 2: AAV8 capsid titration standard curve.*
Various amounts of the AAV8 Reference were run in duplicate. A linear response is seen between $3x10^8$ to $1x10^{10}$ capsids/ml.

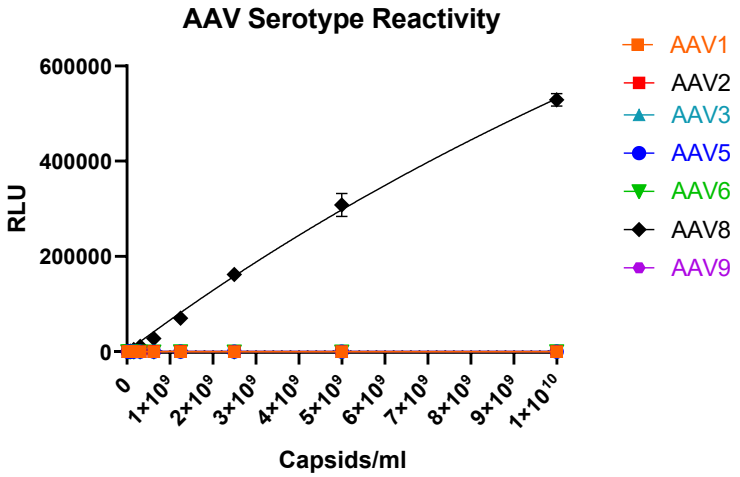


*Figure 3: AAV8 Chemiluminescence ELISA Titration Kit specificity.*
The selectivity of the AAV8 kit was tested against various AAV serotypes. Among all tested AAVs only AAV8 was able to be recognized with this kit.

*Data shown is representative.*

**Troubleshooting Guide**
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

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