

# AAV8

Simple Plex assay for the detection of Adeno-Associated Virus type 8 (AAV8) intact capsids in bioprocess samples.

This assay uses PROGEN's AAV8 (ADK8) antibody.

For research use only. Not for use in diagnostic procedures.

## Sample Preparation

An appropriate dilution factor for each process matrix should be determined experimentally by assessment of sample linearity and spike recovery.

Bioprocess samples require a minimum 2-fold dilution with Sample Diluent SD19 (diluted 1:5). A suggested 2-fold dilution can be achieved by adding 35  $\mu$ L of sample to 35  $\mu$ L of Sample Diluent SD19 (diluted 1:5). Samples above the ULOQ require further dilution.

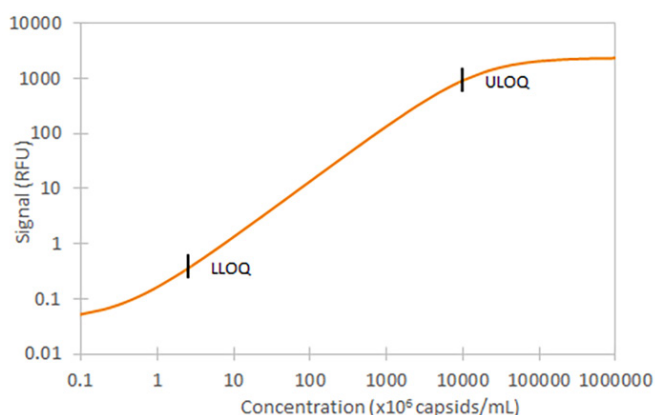
## Reagent Preparation

**Prior to use, allow reagents to reach room temperature.**

**SD19 Concentrate (Diluted 1:5)** - Add 10 mL of SD19 Concentrate to 40 mL of deionized or distilled water to prepare 50 mL of Sample Diluent SD19 (diluted 1:5).

## Calibration Curve

The factory generated calibration curve shown below was compiled by averaging 5 replicates of each calibrator from multiple runs. The 4PL curve fit shows calibrator concentration as a function of signal intensity (relative fluorescent units, RFU).



## Limit of Quantitation

Data shown represents typical performance results for Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) of AAV8.

|      | CONC. (capsids/mL) |
|------|--------------------|
| LLOQ | $2.47 \times 10^6$ |
| ULOQ | $9428 \times 10^6$ |

## Limit of Detection

The limit of detection (LOD) of AAV8 is  $0.10 \times 10^6$  capsids/mL. The LOD was calculated by adding three standard deviations to the mean background signal determined from multiple runs.

## Precision

**Intra-Assay Precision:** Each control was tested 16 times in one assay.

**Inter-Assay Precision:** Replicates of each control were tested in multiple assays performed by at least three technicians using two lots of reagents.

| PARAMETER                              | LOW QC | HIGH QC |
|--|--------|---------|
| Intra-Mean ( $\times 10^6$ capsids/mL) | 23.1   | 1073    |
| Intra-assay SD                         | 0.658  | 55.4    |
| Intra-assay CV (%)                     | 2.9    | 5.2     |
| Inter-Mean ( $\times 10^6$ capsids/mL) | 49.2   | 2202    |
| Inter-assay SD                         | 4.51   | 99.3    |
| Inter-assay CV (%)                     | 9.2    | 4.5     |

## Correlation

This assay has been correlated to the PROGEN ELISA Kit with an  $R^2$  value greater than 0.9.

## Precautions

When handling AAV8 material avoid vortexing and freeze thaw cycles.

## Linearity

Samples containing and/or spiked with high concentrations of AAV8 were serially diluted with Sample Diluent to produce samples within the dynamic range of the assay.

| DILUTION | PARAMETER         | BIOPROCESS<br>(n=9) |
|----------|-------------------|---------------------|
| 1:2      | Avg % of Expected | 97                  |
|          | Range (%)         | 82-117              |
| 1:4      | Avg % of Expected | 87                  |
|          | Range (%)         | 78-106              |
| 1:8      | Avg % of Expected | 89                  |
|          | Range (%)         | 80-106              |
| 1:16     | Avg % of Expected | 89                  |
|          | Range (%)         | 73-111              |

## Specificity

This assay recognizes natural and recombinant AAV8. The AAV8 (ADK8) antibody used in this assay cross-reacts with AAV1, AAV3, AAV7, and AAVrh10.



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