



## AVB Sepharose HP leakage ELISA

Enzyme Linked Immuno-Sorbent Assay for the measurement of Adeno associated virus (AAV) affinity ligand.

### Intended Use

This kit is intended for use in quantifying Adeno associated virus affinity ligand. The kit is for research and manufacturing use only and is not intended for diagnostic use.

### Introduction

AVB Sepharose HP affinity medium (GE Healthcare) is designed for the purification of Adeno associated viruses (AAV) of several subclasses. The AVB Sepharose HP affinity ligand is based on a single chain antibody fragment that was developed by screening for unique selectivity for AAV of subclasses 1, 2, 3, and 5 (CaptureSelect AAV Affinity Ligand). The ligand, a 14 kD recombinant protein, is coupled with multipoint attachment through stable amide bonds to give high chemical stability and low leakage. Even when covalently attached, small amounts of the AAV affinity ligand can leach off of the chromatography support and co-elute with the virus particles. This ELISA provides sensitivity to detect AAV affinity ligand contamination to less than 0.5 ng/ml. This assay is designed to minimize interference and to provide accurate quantitation in the presence of Adeno associated viruses. This kit can be used as a tool to aid in optimal purification process development and in routine quality control of in-process streams as well as final product.

### Principle of the Assay

The AVB Sepharose HP leakage assay is designed to detect AVB Sepharose HP affinity ligand in solution. The assay is a sandwich ELISA, utilizing microtiter plates coated with affinity purified goat anti-AAV affinity ligand. Samples containing AAV affinity ligand are incubated in microtitre plate coated wells. Unbound materials are removed by washing. Bound AAV affinity ligand is detected by biotinylated goat anti-AAV affinity ligand. After incubation excess conjugate is washed out and a streptavidin horseradish peroxidase conjugate is added. This will bind to any of the biotinylated antibody of the sandwich complex. After washing, a substrate reactive with horseradish peroxidase is added (tetramethylbenzidine-hydrogenperoxide). The amount of hydrolyzed substrate is read on a microtiter plate reader and will be directly proportional to the concentration of AAV affinity ligand present. Accurate quantitation is achieved by comparing the signal of unknowns to AAV affinity ligand standards assayed at the same time.



## Provided Materials

- \* **Coating reagents:** 100 µl Goat IgG anti-AVB Sepharose HP affinity ligand
- \* **Standard solution:** 100 µl AVB Sepharose HP affinity ligand
- \* **Biotinylated reagents:** 100 µl Biotinylated Goat IgG anti-AVB Sepharose HP affinity ligand

**Upon arrival all reagents should be stored at -20 °C for stability.**

## Required Materials and Equipment (not provided)

- \* Phosphate buffered saline (PBS) pH 7.4
- \* Tween 20
- \* Bovine Serum Albumin (BSA), Fraction V 99% pure (Sigma-Aldrich)  
**Note:** *Other qualities of Bovine Serum Albumin or other blocking proteins might result in higher background levels.*
- \* Streptavidin-Horseradish Peroxidase
- \* Tetramethylbenzidine (TMB) and Hydrogenperoxide (H<sub>2</sub>O<sub>2</sub>) substrate
- \* 1 M H<sub>2</sub>SO<sub>4</sub>
- \* Microtiter plate (Maxisorp, Nunc)
- \* Microtiter plate shaker
- \* Microtiter plate reader (450 nm)

## Protocols and results

The protocols and data described below were generated by BAC BV to establish the performance and validity of the materials in the ELISA kit. This data is intended to supplement and not to replace user generated validation data. The data is representative of what a laboratory can expect to achieve when the described materials and protocols are used. It is recommended that a user validation study include at least the following experiments to validate this kit with their product: 1) Each user should perform intra and inter assay precision experiments to establish their reproducibility. 2) Each user should perform recovery experiments using their test samples with known amounts of the 500 µg/ml standard solution which is included in the kit.

## Protocol

- Dilute the **Coating reagents** 100 times with PBS pH 7.4.
- Add 100 µl diluted **Coating reagents** to each well and incubate the microtiter plate overnight at 4 °C (39 °F).
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Block the plate for 30 minutes at room temperature with 250 µl/well 4 (w/v)% BSA in PBS pH 7.4 on a microtiter plate shaker.
- Wash the plate 1 time with PBS pH 7.4 + 0.05 (v/v)% Tween 20.



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- Prepare a standard dilution serie by diluting the **Standard solution**. Recommended is 1000 times to 500 ng/ml in dilution buffer containing 2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4, followed by dilutions with a factor three.

1: [500] = 1  $\mu$ l **Standard solution** + 999  $\mu$ l dilution buffer

2: [167] = 300  $\mu$ l [500] + 600  $\mu$ l dilution buffer

3: [ 56] = 300  $\mu$ l [167] + 600  $\mu$ l dilution buffer

Up to

8: [ 0.23] = 300  $\mu$ l [ 0.69] + 600  $\mu$ l dilution buffer

- Dilute 75  $\mu$ l sample with 75  $\mu$ l 2 times concentrated dilution buffer (4 (w/v)% BSA + 0.1 (v/v)% Tween 20 in PBS pH 7.4).

- Add 100  $\mu$ l standard or sample per well.
- Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Dilute the **Biotinylated reagents** 100 times with 2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4.
- Add 100  $\mu$ l diluted **Biotinylated reagents** to each well and incubate the plate 1 hour at room temperature.
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Add 100  $\mu$ l 1:4000 diluted Streptavidin-Horseradish peroxidase in 2 (w/v)% BSA + 0.05 (v/v)% Tween 20 in PBS pH 7.4.
- Incubate the plate 1 hour at room temperature on a microtiter plate shaker.
- Wash the plate 5 times with PBS pH 7.4 + 0.05 (v/v)% Tween 20.
- Wash the plate 2 times with demineralized water.
- Add 100  $\mu$ l 1:1 mixed TMB/H<sub>2</sub>O<sub>2</sub> substrate per well.
- Incubate the plate for approximately 5 minutes on a microtiter plate shaker.
- Stop the coloring reaction with 50  $\mu$ l 1M H<sub>2</sub>SO<sub>4</sub>.
- Measure the OD of the microtiter plate at 450 nm with a microtiter plate reader.

### Calculation of Results

The standards are used to construct a standard curve with values reported in ng/mL. This data reduction may be performed through computer methods using curve fitting routines such as 4 parameter logistic fit. Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies.

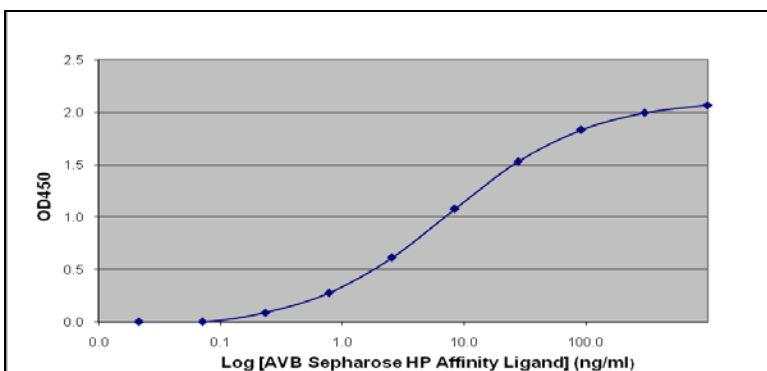


Figure 1: Calibration curve AVB Sepharose HP leakage assay

## Contact

For further information or questions about the AVB Sepharose HP leakage ELISA please send an E-mail to [ligands@captureselect.com](mailto:ligands@captureselect.com)