Avitide AVIPure® – AAV9 Residual Ligand Assay

Immunoenzymetric Competition Assay for the Measurement of Avitide AVIPure® – AAV9 Residual Ligand Catalog # F970

Intended Use

These kits are intended for use in quantitating the Avitide AVIPure® – AAV9 Ligand. These kits are for **Research and Manufacturing Use Only** and are not intended for diagnostic use in humans or animals. The F970 kits incorporate a well qualified sample treatment method to dissociate the ligand from the AAV virus.

Summary and Explanation

AVIPure® – AAV9 Ligand, immobilized on various chromatography media, is used to purify AAV9 virus. Even when covalently attached, the ligand can leach off of the chromatography support and co-elute with AAV. Residual AVIPure® – AAV9 Ligand must be minimized to avoid any adverse patient effects. Our immunoassay method provides sensitivity to the AVIPure® – AAV9 Ligand to less than 1ng/mL. 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. The AVIPure® – AAV9 Ligand assay is not designed to detect all currently marketed constructs used for the purification of AAV virus.

Principle of the Procedure

The Avitide AVIPure® – AAV9 Ligand assay is a competition immunoenzymetric assay. The biotinylated ligand is first bound to the streptavidin coated plate. After a wash step to remove any unbound ligand, the antibody, directly labeled with Horse Radish Peroxidase (HRP), as well as the AVIPure® – AAV9 Ligand is then added to the microtiter plate. After an additional wash step to remove any unbound reactants, the strips are then reacted with tetramethylbenzidine (TMB) substrate. The amount of hydrolyzed substrate is read on a microtiter plate reader and will be directly proportional to the concentration of AVIPure® – AAV9 Ligand present in the sample. Accurate quantitation is achieved by comparing the signal of unknowns to AVIPure® – AAV9 Ligand standards assayed at the same time.

Reagents & Materials Provided

Component	Product
Anti-AVIPure® – AAV9 Ligand:HRP Goat polyclonal antibody conjugated to HRP in a protein matrix with preservative. 1x16mL	F971
Streptavidin coated microtiter strips 12x8 well strips in a bag with desiccant	F972
AVIPure® – AAV9 Ligand Standards AVIPure® – AAV9 Ligand in a protein matrix with preservative. 7 Standards at 0, 0.8, 2, 4, 8, 15, and 30 ng/mL. 1mL/vial	F973
Biotinylated AVIPure® – AAV9 Ligand. Biotinylated AVIPure® – AAV9 Ligand in a protein matrix with preservative 1x11mL	F974
AVIPure® Sample Diluent Tris buffered saline dissociation buffer with a preservative. 1x25mL	I300
Stop Solution 0.5M sulfuric acid. 1x12mL	F006
TMB Substrate 3,3',5,5' Tetramethylbenzidine. 1x12mL	F005
Wash Concentrate (20X) Tris buffered saline with preservative. 1x50mL	F004
Sample Treatment Plate Skirted 96 well PCR plate with adhesive foil seal	F402

Preparation of Reagents

- Bring all reagents to room temperature.
- Dilute wash concentrate to 1 liter in distilled water, label with kit lot and expiration date, and store at 4°C.

Storage & Stability

- All reagents should be stored at 2°C to 8°C for stability until the expiration date printed on the kit.
- Reconstituted wash solution is stable until the expiration date of the kit.
- After prolonged storage, you may notice a salt precipitate and/or yellowing of the wash concentrate. These changes will not impact assay performance. To dissolve the precipitate, mix the wash concentrate thoroughly and dilute as directed in the 'Preparation of Reagents' section.

Materials & Equipment Required But Not Provided

- Microtiter plate reader spectrophotometer with dual wavelength capability at 450 & 650nm. *(If your plate reader does not provide dual wavelength analysis you may read at just the 450nm wavelength.)*
- Pipettors - 50µL and 100µL
- Multichannel pipettor – 25µL, 50µL, and 100µL
- Microtiter plate rotator (400 - 600 rpm)
- Distilled water
- 1 liter wash bottle for diluted wash solution

Precautions

- For Research or Manufacturing use only.
- Stop reagent is 0.5M H_2SO_4. Avoid contact with eyes, skin, and clothing.
- This kit should only be used by qualified technicians.

Procedural Notes

- Complete washing of the biotinylated ligand bound plate to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. The manual wash procedure described below generally provides lower backgrounds, higher specific absorbance, and better precision.

Limitations

- Before reporting the AVIPure® – AAV9 Ligand impurity results, each laboratory should qualify that the kit and assay procedure utilized yield acceptable specificity, accuracy, and precision. A suggested protocol for this qualification can be obtained by contacting our Technical Services Department or at our web site. In general, the most critical qualification experiments involve spike & recovery and dilutional linearity/parallelism.
- Samples in concentrated strong acids can interfere in the assay by lowering the assay pH to below the optimal range of 7.0 to 7.5. The HRP labeled antibody is in a strong buffer designed to neutralize most samples back to the ideal assay pH range.
- **Certain sample matrices and product antibodies may interfere in this assay. Although the assay is designed to minimize matrix interference, materials such as detergents in high concentration, extremes of pH (<6.0 and >8.5), very high buffer molarity, or very high protein concentrations may give erroneous results.** For these reasons we recommend that you first establish acceptable recovery in your sample matrices by performing a spike recovery experiment. **This test can be very simply performed by diluting 1 part of the 30ng/mL standard supplied with the kit into 4 parts of your sample matrix which does not contain any or very low levels of the AVIPure® – AAV9 Ligand. This diluted standard when assayed as an unknown should give a recovery of ~4.8 to 7.2ng/mL. Consult *Cygnus Technologies* Technical Service Department for advice on how to quantitate the assay in problematic matrices.**

Sample Treatment

Sample Treatment Protocol

1. Make sample dilutions and transfer 75 µL to Sample Treatment Plate (STP, Cat#F402).
2. Add 75 µL of the kit standards and controls to STP.
3. Add 150 µL of HRP labeled detection antibody (Cat# F971). Mix by pipetting up and down ~10 times.
4. Incubate on bench for ~5 minutes.

Assay Protocol Notes

- Bring all reagents to room temperature.
- Set-up plate spectrophotometer to read dual wavelength at 450nm for the test wavelength and 650nm for the reference wavelength.
- All standards, controls and samples should be treated in the same way. Assay all samples at least in duplicate.
- Maintain a repetitive timing sequence from well to well for all assay steps to ensure that all incubation times are the same for each well. Accomplish all steps as rapidly as possible to avoid "end of run" sequential process time differences that could cause systematic inaccuracies.
- Make a work list for each assay to identify the location of each standard, control, and sample.
- Thorough washing is essential to proper performance of this assay. The manual method described in the assay protocol was used in development to generate the qualification data for precision, sensitivity and accuracy. A more detailed discussion of this procedure can be obtained from our Technical Services Department or on our web site. In addition, a video demonstration is available in the 'Technical Help' section of our web site.
- The protocol specifies use of an approved orbital microtiter plate shaker for the immunological steps. These can be purchased from most laboratory supply companies. **Do not shake during the 30-minute substrate incubation step, as this may result in higher backgrounds and worse precision.**
- For best results add the HRP conjugate in the same direction as the replicates on the plate. For example, if the replicates are in A1 and A2 position the multichannel pipette horizontally when adding

the HRP conjugate to the standards, samples, and controls.

Assay Protocol

1. Pipette 100µL of Biotinylated AVIPure® – AAV9 Ligand (#F974) to the streptavidin coated microtiter plate wells (#F972).

2. Cover & incubate on orbital shaker at 400-600 rpm for 1 hour at room temperature, 24°C ± 4°C.

3. Dump contents of wells into waste. Blot and gently but firmly tap over absorbent paper to remove most of the residual liquid. Fill wells generously to overflowing with diluted wash solution using a squirt bottle. Dump and tap again. Repeat for a total of 4 washes.

4. Pipette 150µL of the HRP labeled detection antibody + standards, samples and controls (prepared according to "Sample Treatment Protocol") to the wells.

5. Cover & incubate on orbital shaker at 400-600 rpm for 1 hour at room temperature.

6. Repeat Washing Step 3.

7. Pipette 100µL of TMB substrate (#F005) and incubate at room temperature for 30 minutes. DO NOT SHAKE.

8. Pipette 100µL of Stop Solution (#F006).

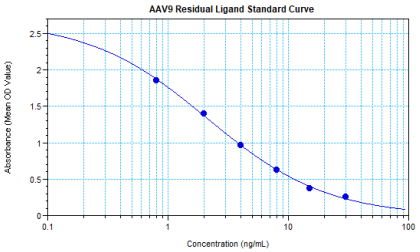
9. Read absorbance at 450/650nm.

Calculation of Results

The standards may be 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 point-to-point, spline, or 4-parameter logistic fit. **Do not use linear regression analysis to interpolate values for samples as this may lead to significant inaccuracies!**

Example Data

F970 Standard Curve			
Well #	**Contents**	**Abs. at 450-650nm**	**Mean Abs.**
A1	Zero Std	2.761	2.675
A2	Zero Std	2.660	
A3	Zero Std	2.605	
B1	0.8ng/mL	1.844	1.852
B2	0.8ng/mL	1.828	
B3	0.8ng/mL	1.885	
C1	2ng/mL	1.447	1.397
C2	2ng/mL	1.340	
C3	2ng/mL	1.403	
D1	4ng/mL	0.988	0.964
D2	4ng/mL	0.940	
D3	4ng/mL	0.965	
E1	8ng/mL	0.627	0.624
E2	8ng/mL	0.626	
E3	8ng/mL	0.620	
F1	15ng/mL	0.360	0.373
F2	15ng/mL	0.378	
F3	15ng/mL	0.380	
G1	30ng/mL	0.253	0.251
G2	30ng/mL	0.250	
G3	30ng/mL	0.249	



Procedural Modifications

Samples containing AVIPure® – AAV9 Ligand greater than 30ng/mL should **only** be diluted in the provided AVIPure® Sample Diluent (Cat # I300). Be sure to multiply diluted sample concentrations by the dilution factor when calculating the results.

Quality Control

- Precision on duplicate samples should yield average % coefficients of variation of less than 20% for samples in the range of 2 – 15 ng/mL. CVs for samples > 15 ng/mL may be greater than 20%.
- It is recommended that each laboratory assay appropriate quality control samples in each run to

ensure that all reagents and procedures are correct. You are strongly urged to make controls in your typical sample matrix using your product antibody. These controls can be aliquoted into single use vials and stored frozen for long-term stability.

Performance Characteristics

Cygnus Technologies has qualified this assay by conventional criteria as indicated below. A more detailed copy of this "Qualification Summary" report can be obtained by request by accessing our web site at www.cygnustechnologies.com. This qualification is generic in nature and is intended to supplement but not replace certain user and product specific qualification and qualification that should be performed by each laboratory. At a minimum, each laboratory is urged to perform a spike and recovery study in their sample types. In addition, any of your sample types containing Avitide AVIPure® – AAV9 Residual Ligand within or above the analytical range of this assay should be evaluated for dilution linearity. Each laboratory and technician should also demonstrate competency in the assay by performing a precision study similar to that described below. A more detailed discussion of recommended user qualification protocols can be obtained by contacting our Technical Services Department or on-line at our web site.

Precision

The data below show both intra (n=20 replicates) and inter-assay (n=10 assays) coefficients of variation (%CVs) for 3 control samples in the low, middle, and upper range of the standards. Each laboratory is encouraged to establish precision with its protocol using a similar study.

F970 Precision		
Intra-assay		
# of tests	**Target (ng/mL)**	**%CV**
20	40	13%
20	6.0	12%
20	2.0	10%
Inter-assay		
# of assays	**Target (ng/mL)**	**%CV**
10	40	6.3%
10	6.0	8.5%
10	2.0	6.3%

Sensitivity

The lower limit of detection (**LOD**) is defined as that concentration corresponding to a signal three standard deviations below the mean of the zero standard. The LOD is ~260 pg/mL. The lower limit of quantitation (**LLOQ**) is defined as the lowest concentration at which recovery is within 20% of the nominal level and the repeatability is within 20% of the CV. The LLOQ of these methods is 800 pg/mL.

Spike & Recovery/Interference Studies

Each user should qualify that their sample matrices and product itself yield accurate recovery. This experiment can be performed by spiking the 30ng/mL standard provided with this kit, into the sample in question. For example, we suggest adding 1 part of the 30 ng/mL standard to 4 parts of the test sample. This yields an added spike of ~6ng/mL. Any endogenous Avitide AVIPure® – AAV9 Ligand from the sample itself, determined prior to spiking and corrected for the 25% dilution of that sample, should be subtracted from the value determined for the spiked sample. The added spike and recovery should be within allowable limits, e.g. 80% to 120%.

Specificity/Cross-Reactivity

Cross-reactivity to non-HCP components has not been extensively investigated with this kit. You should evaluate components in your samples for positive interferences such as cross-reactivity and non-specific binding. Negative interference studies are described in the precision section.

Ordering Information/ Customer Service

Cygnus Technologies also offers kits for the extraction of Host Cell DNA. The following kits are available:

- Residual Host Cell DNA extraction:
  Cat # D100W DNA Extraction Kit in 96 deep well plate
  Cat # D100T DNA Extraction Kit in microfuge tubes

To place an order or to obtain additional product information contact *Cygnus Technologies*:

www.cygnustechnologies.com
Cygnus Technologies, LLC
1523 Olde Waterford Way
Leland, NC 28451 USA
Tel: 910-454-9442
Fax: 910-454-9443

Email for all Order inquiries:
orders@cygnustechnologies.com

Email for Technical Support:
techsupport@cygnustechnologies.com