

AVIPure® dsRNA Affinity Ligand ELISA Kit

User Guide

AVIPure® dsRNA Affinity Ligand ELISA Kit UG 9-EL-0090 – 2

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Customer Support

customerserviceUS@repligen.com

+1 (781) 250-0111

Repligen Corporation

41 Seyon Street
Building 1, Suite 100
Waltham, MA 02453
www.repligen.com

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Abbreviations

dH ₂ O	distilled water
ELISA	enzyme-linked immunosorbent assay
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
LOQ	limit of quantification
N	normality
ng/mg	nanogram per milligram
ng/mL	nanogram per milliliter
nm	nanometer
PBS	phosphate buffered saline
PPE	personal protective equipment
rpm	revolutions per minute
TMB	tetramethylbenzidine
μm	micrometer

1. Introduction

The AVIPure® dsRNA Affinity Ligand ELISA Kit (part number 9-EL-0090) from Repligen provides accurate and precise quantitation of residual AVIPure dsRNA Affinity Ligand during AVIPure dsRNA Affinity Resin purification.

Testing for residual AVIPure dsRNA Affinity Ligand occurs in several different phases of development and commercial manufacturing that may include:

- Process development: leaching characteristics of the resin under specific conditions
- Manufacturing: eluted samples taken throughout several points in the purification process
- Finished product release: document process containment levels and lot-to-lot consistency





This user guide provides general guidance for the use of AVIPure dsRNA Affinity Ligand ELISA Kit. For further optimization or troubleshooting support, please contact your local Repligen Field Application Scientist (FAS). If you need assistance contacting your local FAS, the Customer Service team at Repligen would be happy to help (email: customerserviceUS@repligen.com; phone: 781-250-0111).

2. About This Document

Table 1. Explanation of User Attention Phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

Table 2. Safety Precautions

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eye wear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
WARNING 	Flammable liquid and vapor. <ul style="list-style-type: none">• Keep away from heat/spark/open flame/hot surfaces. No smoking.• Keep container tightly closed.• Ground/bond container and receiving equipment.• Store in a well-ventilated place. Keep cool.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/international regulations.

3. Product Description

The AVIPure dsRNA Affinity Ligand ELISA Kit is supplied with AVIPure dsRNA Affinity Ligand standard and provides accurate quantitation of leached dsRNA Affinity Ligand. The polystyrene microtiter plate provided in this kit is coated with AVIPure anti-dsRNA ligand antibody. Standards and test samples are diluted with phosphate buffered saline with 0.1% Tween 20 (PBST) and incubated with the immobilized antibodies. Captured AVIPure dsRNA Affinity Ligand is then detected by the addition of a biotinylated anti-dsRNA ligand antibody detection probe (Reagent C). The high substitution of the probe allows maximum binding of streptavidin peroxidase conjugate (Reagent D). The final detection step involves adding tetramethylbenzidine (TMB; Reagent E) to give a highly sensitive colorimetric reaction. The color intensity is proportional to the amount of AVIPure dsRNA Affinity Ligand present in the sample.

Table 3. Product Characteristics

Reagent	Description	Volume	Storage
Reagent B	AVIPure dsRNA Affinity Ligand standard solution, concentration of 1.0 mg/mL in sterile water	200 µL	2 – 8°C
Reagent C	Goat anti-dsRNA ligand antibody: Biotin probe, contains 0.02% sodium azide	200 µL	2 – 8°C
Reagent D	Streptavidin-HRP (horseradish peroxidase) conjugate	200 µL	2 – 8°C
Reagent E	TMB peroxidase substrate, contains 3, 3', 5, 5'-tetramethylbenzidine in buffer	20 mL	2 – 8°C
PBS packs	Final volume of each pack when reconstituted is 1 L	2 packs	Ambient
ELISA plate	96-well microtiter plate coated with Goat anti-dsRNA ligand antibody, packed with desiccants	Dried Plate	2 – 8°C

Note: Reagents are specific to the kit lot and should be discarded once all plate strips have been consumed.

The following reagents, supplies, and equipment are not provided with the kit:

- dH₂O or HPLC-grade water (preferred)
- 1 L graduated cylinder
- 1.5 mL Eppendorf® Tubes
- 15 mL and 50 mL plastic centrifuge tubes
- Tween® 20
- Reagent reservoirs
- 5 mL and 10 mL serological pipettes
- Plate sealers
- Filter (0.22 µm) and 1 L bottle
- Phosphoric acid
- Micro-pipettors and 12-channel pipettor
- ELISA plate reader with wavelength capability at 450 nm
- Timer
- Vortex mixer
- Micro-centrifuge
- Water bath

3.1 Pre-assay Reagent Preparation

3.1.1 All Kit Components

Allow all kit components to equilibrate to room temperature ([see note below](#)).

3.1.2 PBS Solution

Dissolve the contents of one PBS pack in 800 mL of dH₂O to a final volume of 1 L. Mix well. Filter PBS solution through a 0.22 µm filter.

3.1.3 PBS-Tween 20 Wash Solution

Pour 700 mL of the PBS solution (prepared and filtered per instructions above) into a 1 L graduated cylinder. Add 700 µL of Tween 20. Mix well. Save the remaining 300 mL PBS solution for the final ELISA wash. Filter PBS-Tween solution through a 0.22 µm filter.

3.1.4 TMB Substrate Solution

For a full-plate assay, use the whole bottle of TMB. For a half-plate assay, aliquot 8 mL of TMB into a 15 mL conical centrifuge tube and cover with aluminum foil to protect from light. Return bottle to a 2 – 8°C refrigerator.

3.1.5 Test Samples

Allow all test samples to equilibrate to room temperature (see note below).

Note: An ideal room temperature range of 65 – 77°F (18 – 25°C) is important for optimum assay performance.

3.2 Sample Preparation

Prior to running the assay, dilute AVIPure dsRNA Affinity Ligand-purified RNA samples into phosphate buffered saline (PBS) with 0.1% Tween 20 to reach a final concentration ≤0.5 mg/mL. For best performance, characterize the assay with process-specific buffers and sample.

Note: If sample concentration is too low and further dilution is a concern, sample can be directly loaded to ELISA plate with 0.1% Tween present.

3.3 Standard Preparation

1. When Reagent B reaches room temperature, mix by vortex. If reagent remains on the sides or cap of the tube, briefly spin in a micro-centrifuge.
2. Label three 1.5 mL Eppendorf tubes as Tube 1, Tube 2, and Tube 3. Prepare the standard solutions by diluting Reagent B with 1X sample diluent ([Table 4](#)). Vortex each tube thoroughly between dilutions. Low protein binding tubes should be used.
3. Place Tube 3 (2 ng/mL AVIPure dsRNA Affinity Ligand standard) aside.

Note: Standard curve points may be added at lower concentration to extend range.

Table 44. Concentrated Standard Solution Preparation

Tube	Volume Reagent B (AVIPure dsRNA Affinity Ligand Standard)	Volume 1X Sample Diluent
1	10 µL of Reagent B	990 µL
2	10 µL of Tube 1	990 µL
3	20 µL of Tube 2	980 µL

3.4 Plate Set-up

These instructions describe preparation of a 7-point standard curve in triplicate. Two-fold dilution series of the standard curve and protein samples are used starting in wells H1 – H3 and D4 – D6, respectively.

Note: The following pipetting and suggested dilution instructions are specific to a single sample assay ([Table 5](#)). Analogous steps should be taken when performing the assay according to your personal design. Alternatively, users may choose to prepare standards and samples in a dilution plate and transfer to an assay plate. A low protein binding dilution plate is recommended.

- Using a 12-channel pipettor, add 100 µL of PBST into wells A4 – A6, B1 – B6, C1 – C6, D1 – D3, E1 – E3, F1 – F3, and G1 – G3.
- Transfer 200 µL of 2 ng/mL AVIPure dsRNA Affinity Ligand standard solution (Tube 3) into wells H1 – H3.
- Transfer 200 µL of RNA sample into wells D4 – D6.
- Make 2-fold serial dilutions of the AVIPure dsRNA Affinity Ligand standard and RNA samples by transferring 100 µL from each set of triplicate wells into the wells directly above them. Mix thoroughly by pipetting 5 times.

Note: In a single sample assay format, the same tips can be used for each row.

- After making the last AVIPure dsRNA Affinity Ligand standard serial dilution in wells C1 – C3, remove 100 µL and discard. Also discard 100 µL from the final RNA sample dilution in wells A4 – A6.

Table 55. Representative Plate Set-up for One RNA Sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	Plate blank			1:8								
B	0 ng/mL			1:4								
C	0.0625 ng/mL			1:2								
D	0.125 ng/mL			Sample #1								
E	0.25 ng/mL											
F	0.5ng/mL											
G	1 ng/mL											
H	2 ng/mL											

3.5 ELISA Procedure

- After the AVIPure dsRNA Affinity Ligand standards and RNA sample dilutions have been added to the plate, cover the plate and incubate at room temperature for 30 minutes.
- After incubation, remove all liquid from the wells. Using a wash bottle or automated plate-washing system, wash the plate with PBS-Tween 20 solution. Remove the liquid and dry thoroughly by inverting the plate on clean paper towels and tapping gently. Repeat the wash and dry cycle three (3) more times for a total of four (4) washes.
- Prepare the Goat anti-dsRNA ligand Ab (Reagent C) biotin probe solution:
 - Briefly vortex the Reagent C vial. If reagent material remains on the sides or cap of the tube, briefly spin in a micro-centrifuge.
 - For a full-plate assay, prepare 12 mL of rabbit anti-Protein A biotin probe solution by combining 70 µL of Reagent C with 12 mL of prepared PBS-Tween 20 in a 15 mL conical centrifuge tube. Mix solution thoroughly.
 - For a half-plate assay, prepare 6 mL of rabbit anti-Protein A biotin probe solution by combining 35 µL of Reagent C with 6 mL PBS-Tween 20 in a 15 mL conical centrifuge tube. Mix solution thoroughly.
- Using a 12-channel pipettor, add 100 µL of the diluted Reagent C probe solution to each well containing a test sample or standard. Leave wells A1 – A3 (plate blanks) empty.
- Cover the plate and incubate at room temperature for 30 minutes. After incubation, wash the wells four (4) times with PBS-Tween 20 and remove the liquid. Dry thoroughly by inverting the plate on clean paper towels and tapping gently.

6. Prepare the streptavidin horseradish peroxidase conjugate solution (Reagent D):
 - a. Briefly vortex the Reagent D vial. If reagent material remains on the sides or cap of the tube, briefly spin in a micro-centrifuge.
 - b. For a full-plate assay, prepare 12 mL of streptavidin horseradish peroxidase conjugate solution by combining 12 µL of Reagent D with 12 mL of prepared PBS-Tween 20 in a 15 mL conical centrifuge tube. Mix solution thoroughly.
 - c. For a half-plate assay, prepare 6 mL of streptavidin horseradish peroxidase conjugate solution by combining 6 µL of Reagent D with 6 mL PBS-Tween 20 in a 15 mL conical centrifuge tube. Mix solution thoroughly.
7. Add 100 µL of the diluted Reagent D conjugate solution to each well containing test sample or standard. Leave wells A1 – A3 (plate blanks) empty.
8. Cover the plate and incubate at room temperature for 30 minutes.
9. After incubation, discard the conjugate solution from the plate. Wash the wells twice with PBS-Tween 20. Wash twice more but with PBS only. After each wash, discard the liquid by inverting the plate on clean paper towels and tapping gently.

Note: Before proceeding with the next step, make sure the TMB solution is at room temperature 65 – 77°F (18 – 25°C). If the lab is too warm, move the assay to a cooler location for the development step.

10. Using a multi-channel pipettor, add 100 µL of the TMB substrate to each of the wells, including A1 – A3 (plate blanks).
11. Incubate plate for 5 ± 1 minutes. Stop reaction by adding 100 µL of 1 N phosphoric acid to each well, including A1 – A3 (plate blanks), in the same order of pipetting used for the TMB substrate solution.

Note: Other strong acids typically used as stop solutions in ELISA may be substituted for 1 N phosphoric acid. If bubbles are present in the wells, agitate slightly before reading.

12. Read the plate at 450 nm.

3.6 Important Points Regarding Assay Sensitivity

1. Numerical results of this assay are expressed as nanograms per milliliter (ng/mL) of AVIPure dsRNA Affinity Ligand.
2. The sensitivity of the assay is typically about 0.1 ng/mL.

Assay characterization recommendations are available in Repligen Technical Notes. Please contact Customer Service for a copy or visit repligen.com.

Calculation of Results

1. Calculate the mean absorbance value for the plate blank wells (A1 – A3) and subtract from all remaining wells on the plate (including the 0 ng/mL standard curve.) Determine the average absorbance value for each standard concentration and all test samples.

Note: Method of calculation for standard curve should be based on internal standards. Other curve fits may be used as deemed appropriate.

2. Calculate the standard curve:

Figure 1. Representative AVIPure dsRNA Affinity Ligand Standard Curve by Quadratic Fitting

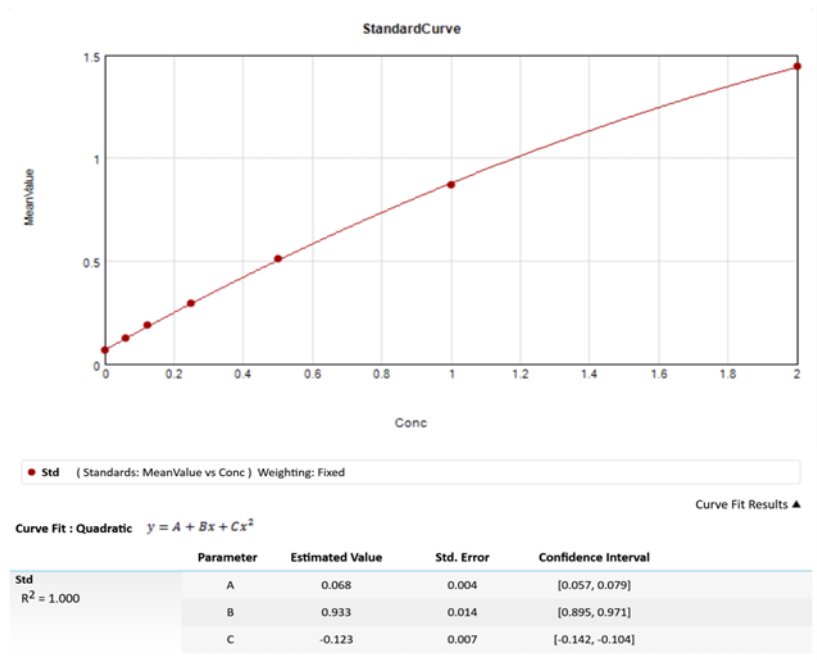
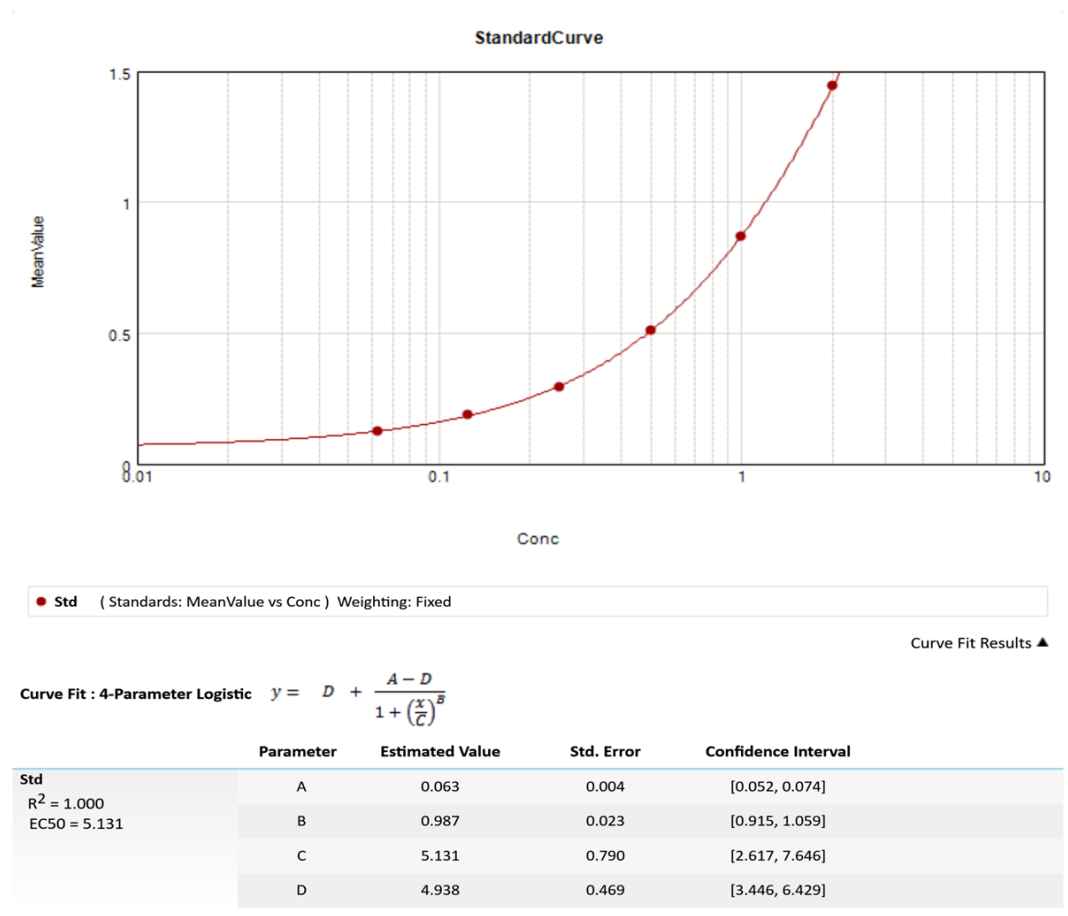


Figure 2. Representative AVIPure dsRNA Affinity Ligand Standard Curve by 4-Parameter Logistic Fitting



The standard curve points can be fitted using curve-fitting software. Model fits such as quadratic or 4--parameter logistical equations are recommended. Such a fit is the acknowledged reference model for sigmoidal immunoassay data (1, 2).

The regression line can be used to determine the AVIPure dsRNA Affinity Ligand concentration for the samples.

$$\text{Sample AVIPure dsRNA Affinity Ligand Conc. (ng/mL)} = \text{each well AVIPure dsRNA Affinity Ligand Conc. Calculated from standard curve} \times \text{Sample Dilutions}$$

To determine the ng/mg (ppm) of AVIPure dsRNA Affinity Ligand per sample, use the following formula:

$$\text{ng/mg} = \frac{\text{Mean Sample AVIPure dsRNA Affinity Ligand Concentration [ng/mL]}}{\text{Sample Concentration [mg/mL]}}$$

4. Troubleshooting

Problem	Possible Cause	Remedy
Not enough of required reagent.	Splashing of reagent on sides or cap of reagent tube during mixing, shipping, or handling.	Briefly centrifuge tube.
Inconsistent results between sample dilutions.	RNA sample was not fully equilibrated in PBST, pH 7.0 – 7.4, before assay.	Ensure pH is 7.0 – 7.4 and re-run assay.
	The RNA sample concentration in the well was >0.5 mg/mL	Ensure sample concentration is diluted in PBST to ≤0.5 mg/mL
Outliers, where one replicate has an abnormally high or low absorbance value.	Small amount of peroxidase conjugate left on the plate before color development (i.e., wells were not thoroughly washed).	Discard outliers and average duplicates. Ensure thorough washing in any subsequent ELISA testing.
Color development time to reach 0.9 AU is >6 minutes.	TMB solution (Reagent E) was not at room temperature before adding to wells.	Solution can be warmed before adding to wells. Use incubator set at 65 – 77°F (18 – 25°C) for all incubations or develop longer than 6 minutes. Note: Absorbance of <0.9 is acceptable if overall signal to noise ratio is ≥6.
	Room temperature too low, or too cool.	
Background signal is >0.150.	Color development for TMB substrate was >6 minutes.	Start timer immediately after adding TMB substrate to 2ng/mL standard wells.
	Temperature of TMB substrate >77°F (25°C).	Store TMB in a location that is 65 – 77°F (18 – 25°C) until use.
	Insufficient plate washing.	Ensure plate was washed 4 times.
O.D. values consistently high for all samples or low recovery of AVIPure dsRNA Affinity Ligand in samples.	Buffer component interference.	Perform a greater fold dilution for sample and ensure 0.1% Tween is present.

5. References and Additional Resources

5.1 Journal Articles

1. Dudley, R.A., P. Edwards, et al. (1985) "Guidelines for immunoassay data processing." Clin Chem 31(8): 1264-71.
2. Smith, W.C. and G.S. Sittampalam (1998) "Conceptual and statistical issues in the validation of analytic dilution assays for pharmaceutical applications." J Biopharm Stat 8(4): 509-32.
3. H. Fey and G. Burkhard, (1981) "Measurement of Staphylococcal Protein A and Detection of Protein A-Carrying Staphylococcus Strains by a Competitive ELISA method" J. Immunol. Methods 47: 99-107.
4. Warnes, A. Walkland and J.R. Stephenson, (1986) "Development of an Enzyme-Linked Immunosorbent Assay for Staphylococcal Protein A Produced in Escherichia coli by pUC8-based Plasmids Containing the Staphylococcus aureus Cowan I protein A Gene" J. Immunol. Methods 93:63-70.
5. M.T. Dertzbaugh, M.C. Flickinger and W.B. Leberer III, (1985) "An Enzyme Immunoassay for the Detection of Staphylococcal Protein A in Affinity-Purified Products" J. Immunol. Methods 83: 169-177.
6. J.W. Bloom, M.F. Wong and G. Mitra, (1989) "Detection and Reduction of Protein A Contamination in Immobilized Protein A-Purified Monoclonal Antibody Preparations" J. Immunol. Methods 117: 83-89.
7. S.M. Knicker, A.T. Profy, (1991) "Immunoassay to Measure Staphylococcal Protein A in the Presence of Murine Immunoglobulins" J. Immunol. Methods 142: 53-59.

5.2 Online Resources

User Guides and Safety Data Sheets are available on the Repligen website, [repligen.com](https://www.repligen.com).

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Customer Service

Repligen Corporation
41 Seyon Street
Waltham, MA, USA 02453

customerserviceUS@repligen.com

(781) 250-0111

