AVIPure® dsRNA Clear OPUS® Column

User Guide

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Abbreviations

µg	microgram
µL	microliter
µm	micron
CIP	clean-in-place
circRNA	circular RNA
cm	centimeter
CV	Column Volume
dsRNA	double-stranded RNA
dT	deoxythymidine
FLuc	firefly luciferase RNA
g	gram
Gu-HCl	guanadine hydrochloride
L	liter
M	molar
mg	milligram
min	minute
mL	milliliter
mM	millimolar
mRNA	messenger RNA
NaCl	Sodium chloride
nm	nanometer
OPUS	Open Platform User Specified

PPE	Personal protective equipment
saRNA	self-amplifying RNA
sec	second
ssRNA	single-stranded RNA

1. Introduction

AVIPure® dsRNA Clear OPUS® Columns remove double-stranded RNA (dsRNA) byproducts from in vitro transcribed mRNA, saRNA, and circRNA. The capacity for dsRNA is ≥0.4 mg per mL of column volume (CV). A 1 mL AVIPure dsRNA Clear OPUS Column can process >10 mg of single-stranded RNA (ssRNA) dependent on dsRNA content.

2. About This Document

This manual uses several different phrases. Each phrase should draw the following level of attention:

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.

3. Safety Precautions

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.
IMPORTANT 	This product is shipped in an 18.0 ±1% ethanol solution, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use. Follow all local regulations for safe disposal.
WARNING 	Flammable liquid and vapor. • 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.
IMPORTANT 	For a full list of precautionary statements, please read the Safety Data Sheet (SDS).

4. Key Performance Attributes

- High specificity of the affinity ligand provides effective dsRNA binding and low non-specific binding
- Simple flow-through process with no organic solvent requirement
- High salt conditions may enable dsRNA removal prior to poly-dT chromatography
- Compatible with standard bioprocess columns and relevant process flowrates

Process Conditions

Optimal conditions for each RNA sample must be determined empirically. dsRNA removal is enhanced with high salt (>0.5 M NaCl) and pH values of pH 7.2 or higher. The level of dsRNA byproducts in a given RNA can vary by several orders of magnitude; therefore, the column should be loaded with approximately 10 mg RNA/mL_{CV} for initial testing. Fractions should be analyzed for dsRNA breakthrough to determine the appropriate ssRNA challenge for a given feed. Depending on the feed, the column may be able to clear significantly more than 10 mg RNA/mL_{CV}, but loading the column to a 10% breakthrough capacity might require loading hundreds of column volumes (in the case of a low dsRNA feed) and long processing times. To avoid this, the column should be sized to allow processing in the desired time frame (e.g., 30 – 60 minutes).

The pressure limit of the resin is 3 bar. The resin should be operated at a flow velocity that maintains a pressure drop across the column lower than 3 bar. ***Factors that influence maximum flow velocity include viscosity of running buffer and presence of insoluble material in the load solution***. The maximum flow velocity will vary depending on the feed stream. When loading 5 cm bed height columns with IVT solutions, the evaluation should be started at 600 cm/hr. IVT solutions should be free of insoluble precipitates to avoid column fouling Filtration is recommended for IVT feeds that have visible precipitates.

Users can increase flow velocity provided the pressure drop over the column stays below 3 bar, up to a maximum of 1200 cm/hr. When loading the resin with purified mRNA, faster flow velocities are possible, up to a maximum of 1200 cm/hr.

Table 3. Process Steps

Step	Column Volumes	Residence Time (Min)	Suggested Buffer	Notes
Sanitization (OPTIONAL)	15	1	Choose one: • 6M guanidine hydrochloride • 0.1 M phosphoric acid • 1 M acetic acid • 100 mM NaOH • 70% Ethanol	A 15 min static hold can also be used for this step
Equilibration	2 – 5	1	Match oligo-dT load buffer, OR 50 mM HEPES, 0.7 M NaCl, 2 mM EDTA, pH 7.2	pH and conductivity return to baseline
Load	>5	1	Adjust RNA to 0.5 – 1.0M NaCl, with buffer pH 7.2 or higher.	Filter RNA solution after adjusting to final pH and conductivity. Challenge with ≥10 mg/mL_{CV}. Monitor sample for time-dependent precipitation
Chase	5	1	Equilibration buffer	-
Elution/CIP	5	1	6 M guanidine hydrochloride	dsRNA elutes and column is regenerated
Re-equilibration	2 – 5	1	Equilibration buffer	pH and conductivity return to baseline
Long-term storage	3	1	18 – 20% ethanol	at 2 – 8° C

The quality of the RNA sample is an important factor in successful processing. Samples that display a single crisp band on a gel or CE trace give the best results (i.e., >2 log reduction of dsRNA). Samples that are smeary, degraded, or display multiple bands may have lower dsRNA clearance. For the best results, optimize the IVT (in vitro transcription) parameters until a feed has a single, sharp RNA band.

Initially, use purified RNA suspended (or diluted) in the oligo dT binding buffer used to purify the RNA. Oligo-dT binding buffers have high salt (>0.5 M), and compatibility with the specific RNA should already be established. Processing purified RNA in this manner should provide the highest dsRNA clearance (>2 log reduction of dsRNA). Some RNAs precipitate slowly (over ~15 – 60 minutes) in high-salt buffers after initially appearing clear and passing through a 0.22 µm filter. This precipitation reduces the yield of RNA in the flow-through and increases the RNA that elutes in 6 M guanidine hydrochloride. This may be avoided by decreasing the salt, by mixing the RNA and salt immediately before the column using inline dilution techniques, by keeping the sample at room

temperature (not on ice or at 4 °C), or by heating the sample (~30 – 37°C) to increase solubility. Because different RNA molecules have different solubility profiles, these approaches need to be tailored for individual RNAs.

To determine the feasibility of situating the AVIPure dsRNA Clear OPUS Column prior to oligo dT purification, perform a crossover study with crude IVT mixtures to determine if any components of the crude IVT reaction interfere with the AVIPure dsRNA Clear OPUS Column. Ensure the solution is filtered before loading the column, as some components of the IVT mixture may precipitate in high salt. When using the AVIPure dsRNA Clear OPUS Column in line with an oligo dT step, bypass the AVIPure column during the oligo dT wash and elution steps.

4.1 Sample Volumes and Column Loading

Because the column operates in flow-through mode, the sample volume should be at least several times larger than the column volume. RNA concentrations between 0.25 and 1 mg/mL are common, and higher concentrations can be tested if desired. Attempting to process very small sample volumes (<5 CV) can lead to very dilute samples due to peak broadening as there is no bind/elute step to concentrate the sample. The sample can be applied by pump or sample loop.

To test the recommended 10 mg mRNA/mL$_{CV}$ in a 1 mL column, 10 mg of mRNA are required. With 0.5 mg/mL mRNA, the sample volume would be 20 mL, and at 1 mg/mL mRNA the sample volume would be 10 mL. Using lower quantities and volumes of RNA can lead to lower apparent recoveries due to excessive dilution from peak broadening, and non-specific binding to system tubing and fraction vessels.

To evaluate larger challenges (e.g., 30 – 100 mg mRNA/mL$_{CV}$), the sample volume can be increased. Alternatively, more concentrated samples can be evaluated. In general, the yield of dsRNA-depleted RNA will increase as the challenge increases. It is important to collect fractions to determine if dsRNA breakthrough occurs with increased challenge.

4.2 Equilibration and Binding Conditions

Binding of dsRNA in AVIPure dsRNA Clear OPUS Columns is optimal with 0.5 – 1.0 M NaCl and pH values of 7.2 or higher. The equilibration should match the composition of the RNA to be processed. Common biological buffers (HEPES, phosphate, tris, etc.) can be used interchangeably. If satisfactory results are not obtained with oligo dT binding buffer, it is recommended to increase the salt and/or pH and retest. This process may be more efficient in filter plate format with loose resin (See related protocol).

4.3 Elution Conditions

The bound dsRNA can be eluted with 6 M guanidine hydrochloride (GuHCl); this buffer also cleans and regenerates the column for reuse. The resin has been held for a total of 20 hours in 6 M GuHCl without loss in dsRNA capacity.

The GuHCl should be removed from the eluate before analytical testing.

Alternatively, 0.1 M NaOH can be used to elute bound dsRNA, but the RNA will be degraded and not suitable for analysis. Prolonged exposure to 0.1 M NaOH (>2.5 hours) may decrease column performance.

4.4 Sanitization and Clean-in-place (CIP) Conditions

Testing indicates that AVIPure dsRNA affinity resin is stable for over 2.5 hours in 0.1 M phosphoric acid, 1 M acetic acid, 70% ethanol, and 0.1 M NaOH. The resin is stable for at least 20 hours in 6 M GuHCl. No loss in capacity is observed in resin exposed to 0.5 M NaOH for up to 30 minutes.

Using 6 M GuHCl as an elution/CIP is recommended, adding additional CIP steps only if necessary. Only 6 M GuHCl and 0.1 M NaOH are highly effective at removing bound dsRNA, and one of these solutions should be used if the column will be cycled.

4.5 Storage

Store columns in 18 – 20% ethanol at 2 – 8° C. Benzyl alcohol is not recommended because it absorbs at 260 nm and can interfere with RNA quantitation.

4.6 Neutralization

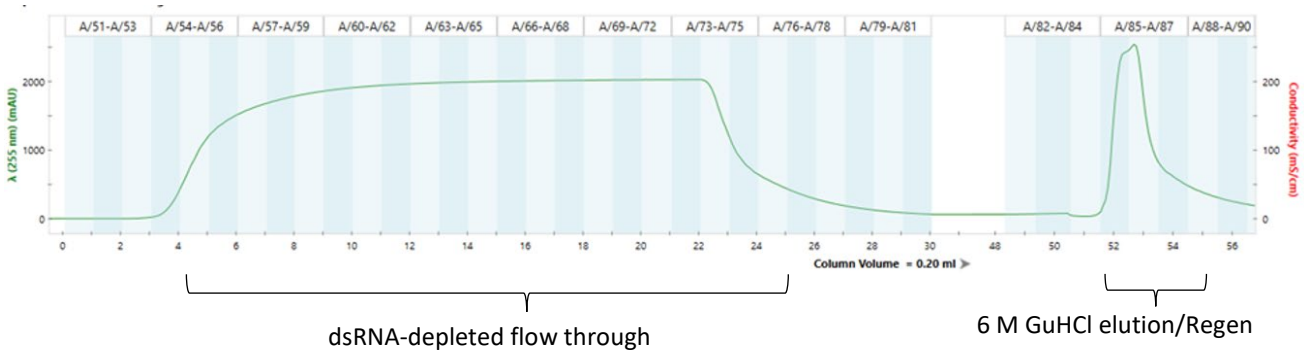
With higher pH values, neutralization buffer should be added to the post-column fractions. This should be tested to ensure compatibility with the specific RNA. A 10% volume of 1 M sodium acetate, pH 4 can be used to neutralize samples run with 50 mM of pH 9.5 carbonate running buffer.

4.7 Example Chromatogram

The absorbance trace on a chromatogram will show a broad peak during loading as the mRNA is flowing through (Figure 1).

- Column: 0.2 mL AVIPure dsRNA Clear OPUS Column
- Buffer: 10 mM Tris pH 8, 0.5 M NaCl, 0.5 mM EDTA
- Elution/CIP: 6 M guanidine HCl
- Flow rate: 0.4 mL/min (30 sec RT)
- Feed: 0.51 mg/mL FLuc with 1.4% dsRNA (by ELISA)
- Challenge: 10.2 mg FLuc/mL$_{CV}$
- Sample application method: Dynamic loop
- Sample volume: 20 CV

Figure 1. Example Chromatogram



5. Ordering Information

Contact your account manager to place an order, or contact your regional customer service using the email addresses below:
North America: customerserviceUS@repligen.com
Europe: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

Table 4. AVIPure dsRNA Clear residual ligand ELISA kit

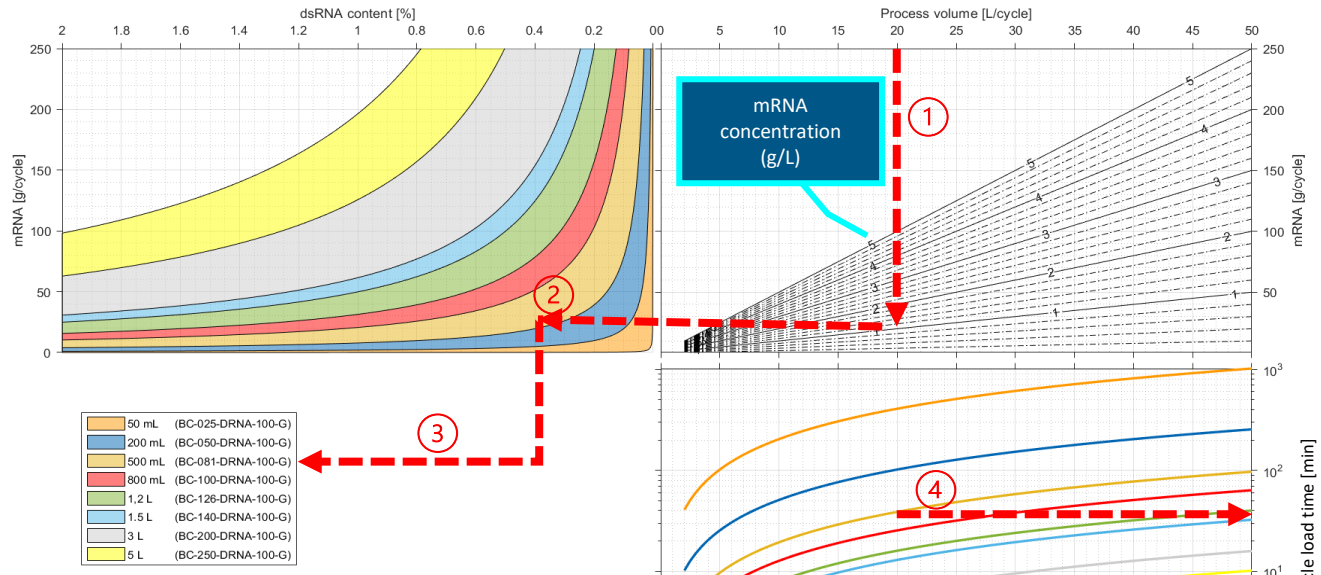
Part #	Description
9-EL-0090	AVIPure dsRNA Clear Affinity Ligand ELISA Kit

Table 5. AVIPure dsRNA Clear Affinity Resin - Bulk

Volume	Part #	Format	dsRNA capacity	Approx. mRNA to load	Notes
5 mL	100RNA-5	5 mL loose resin in 18% EtOH	0.4 mg dsRNA per mL of resin	10 mg per mL of resin	Ideal for high throughput screening in 96-well plate format or small drip columns

Table 6. AVIPure dsRNA Clear OPUS Columns (see sizing guide in Figure 2)

Column Volume (mL)	Part #	Format	dsRNA capacity	Approx. mRNA to load	Notes
8 x 200 µL	23052208R	Strip of 8 Robocolumns®	80 µg dsRNA per column	2 mg per column	For use with Tecan fluid management system for rapid process development
8 x 600 µL	23052208R-30	Strip of 8 Robocolumns	240 µg dsRNA per column	6 mg per column	
0.5 mL	23052205	0.5 x 2.5 cm Minichrom® column	0.2 mg	5 mg	For small scale process development
1 mL	23052206	0.5 x 5 cm MiniChrom column	0.4 mg	10 mg	
5 mL	23052204-100	0.8 x 10 cm MiniChrom column	2 mg	50 mg	
10 mL	23052207-100	1.13 x 10 cm MiniChrom column	4 mg	100 mg	
50 mL	23052210V-100	2.5 x 10 cm Valichrom® column	20 mg	500 mg	Excellent tool for scale up and process validation
50 mL	BC-025-DRNA-100-G	2.5 cm ID x 10 cm bed height	20 mg	500 mg	Process 3 L per hour
200 mL	BC-050-DRNA-100-G	5 cm ID x 10 cm bed height	80 mg	2 g	Process 12 L per hour
500 mL	BC-081-DRNA-100-G	8.1 cm ID x 10 cm bed height	200 mg	5 g	Process 31 L per hour
800 mL	BC-100-DRNA-100-G	10 cm ID x 10 cm bed height	320 mg	8 g	Process 47 L per hour
1200 mL (1.2L)	BC-126-DRNA-100-G	12.6 cm ID x 10 cm bed height	480 mg	12 g	Process 75 L per hour
1500 mL (1.5 L)	BC-140-DRNA-100-G	14 cm ID x 10 cm bed height	600 mg	15 g	Process 92 L per hour
3000 mL (3.0 L)	BC-200-DRNA-100-G	20 cm ID x 10 cm bed height	1200 mg (1.2 g)	30 g	Process 188 L per hour
5000 mL (5.0 L)	BC-250-DRNA-100-G	25 cm ID x 10 cm bed height	2000 mg (2.0 g)	50 g	Process 295 L per hour



How to use the sizing guide:
1) Find the volume and concentration of mRNA to be processed per cycle.
2) Move horizontally to the left to the %dsRNA (w/w) in the RNA feed.
3) Size is indicated by color.
4) Estimate cycle load time (using 1-min residence time) based on volume per cycle and column size.

Figure 2. AVIPure dsRNA Clear column sizing guide – up to 50 L scale

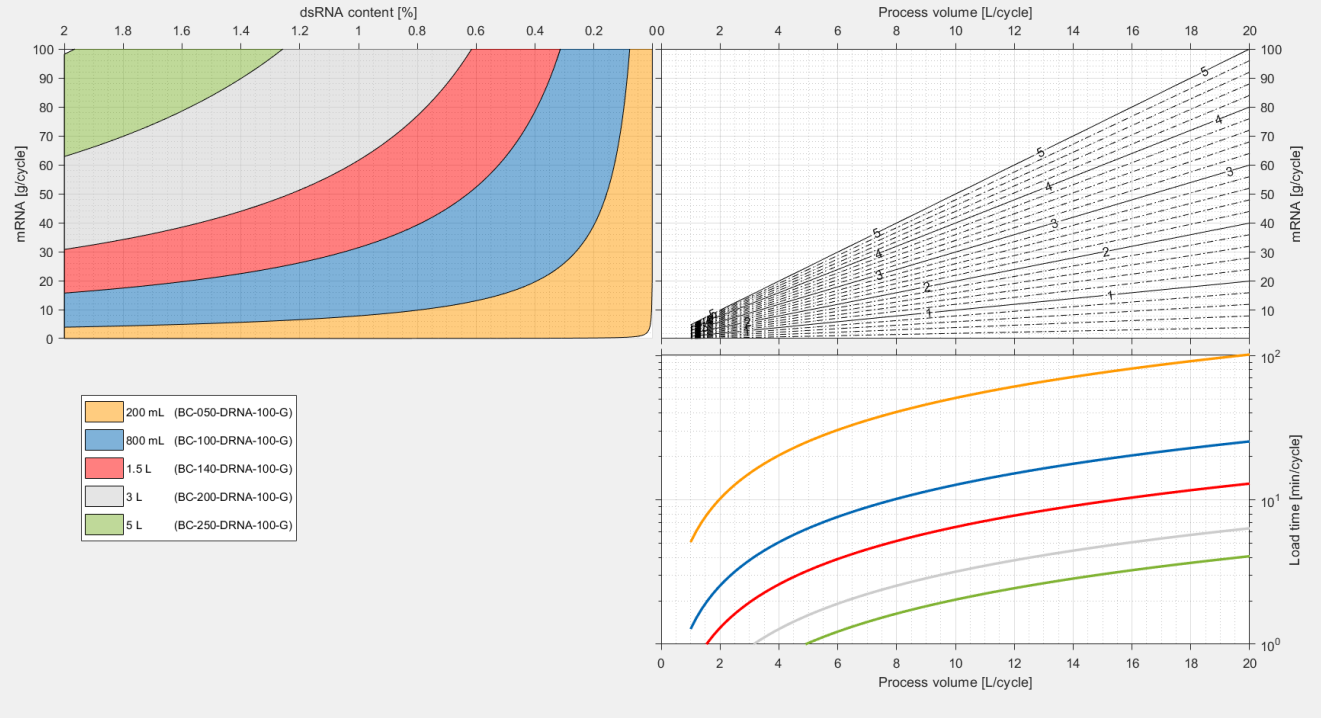


Figure 3. AVIPure dsRNA Clear column sizing guide - up to 20 L process scale

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