Solvent-free removal of double-stranded RNA contaminants from mRNA

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Introduction

Double-stranded RNA (dsRNA) is an unwanted byproduct of in vitro transcription of mRNA. dsRNA is highly immunogenic and co-elutes with mRNA though poly-dT affinity chromatography.

Removal of dsRNA is possible with ion-pair reverse-phase HPLC (RP-HPLC), or cellulose-chromatography in the presence of 16% ethanol. However, these methods are burdensome and expensive because they rely on organic solvents and toxic ion-pairing reagents.

AVIPure® dsRNA is a dsRNA specific scavenger resin that completely removes dsRNA from mRNA feed under aqueous conditions.

A simple flow-through operation removes dsRNA in <30 minutes without affecting mRNA yield.

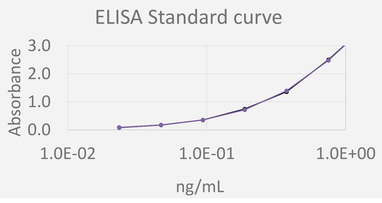
Analytical methods for dsRNA

The dsRNA specific J2 mAb is widely used to assess the dsRNA content of mRNA feeds with immuno-dot blots.

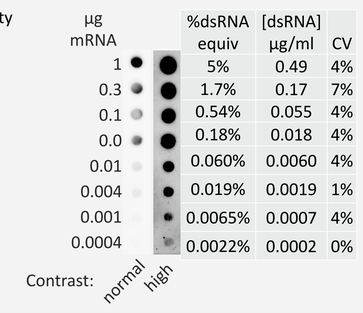
A quantitative dsRNA ELISA kit offers equal sensitivity to the J2 dot blot.

ELISA LoQ = 0.2 ng/ml

J2 dot blot LoQ = 0.002% dsRNA (1 µg spot)



Comparison of J2 dot blot and quantitative ELISA



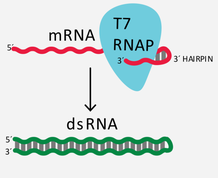
µg mRNA	%dsRNA equiv	[dsRNA] µg/ml	CV
1	5%	0.49	4%
0.3	1.7%	0.17	7%
0.1	0.54%	0.055	4%
0.0	0.18%	0.018	4%
0.01	0.060%	0.0060	4%
0.004	0.019%	0.0019	1%
0.001	0.0065%	0.0007	4%
0.0004	0.0022%	0.0002	0%

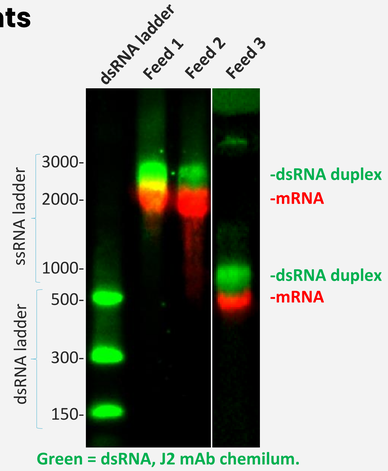
Size of dsRNA contaminants

As a minor contaminant, dsRNA size cannot be determined with stained gels or HP-SEC chromatography.

We developed an immuno-northern blot using the J2 mAb to measure the size distribution of dsRNA.

In three mRNA feeds, the dsRNA contaminants are duplex mRNA, formed by extension of 3' hairpin structures (ref. 1,2).





Green = dsRNA, J2 mAb chemilum.
Red = total RNA, SyberSafe/UV
1.5% agarose gel

AVIPure® dsRNA binds small and large dsRNAs

Experiment:

Challenge AVIPure resin with a mixture of purified dsRNAs and with mRNA feed. Compare to cellulose/ethanol chromatography (ref. 3).

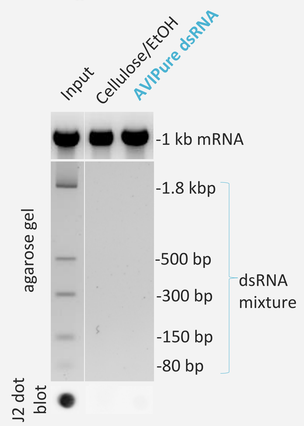
Result:

AVIPure binds all sizes of dsRNA assayed, from 80-1,800 bp (molecular mass of 1,150 kD).

The flow-though had no signal by J2 dot blot.

No mRNA binding was observed.

AVIPure dsRNA matches the performance of cellulose/ethanol chromatography.



AVIPure outperforms RP-HPLC and cellulose/ethanol chromatography with mRNA feed

Experiment:

Flow mRNA feed though AVIPure resin. Compare to RP-HPLC and cellulose/ ethanol.

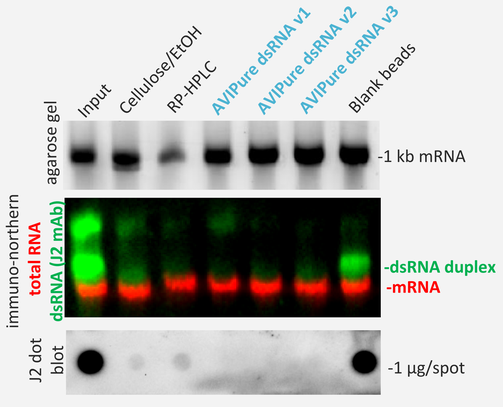
mRNA challenge = 2.5 g/L_{res}

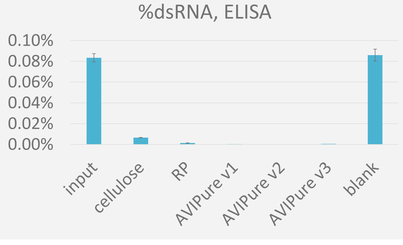
dsRNA challenge = 0.002 g/L_{res}

Result:

AVIPure reduced dsRNA to levels that were undetectable by J2 dot blot or ELISA, a >270-fold reduction in dsRNA.

The immuno-northern blot confirms that mRNA duplexes are completely removed





Method	Fold Reduction (ELISA)
Cellulose/EtOH	13
RP-HPLC	58
AVIPure	**>270**

AVIPure dsRNA scavenger resin

- Flow though application
- Single pass, single use, pre-packed
- Process time <30 minutes
- Available in R&D or cGMP grades

Parameter	Target Specification
Binding Capacity for dsRNA	>1 mg/mL
Residual dsRNA	<0.002%
Operating Flow rates/Residence time	2 - 4 min
Step Yield of mRNA	>95%
Dilution factor (Volume increase)	<10%

R&D, PD Scale	
mRNA Quantity treated	Device size
0.1 g	1 mL
1 g	10 mL

cGMP Now	
mRNA Quantity treated	Device size
10 g	100 mL
100 g	1 L
0.5 kg	5 L

cGMP Large	
mRNA Quantity treated	Device size
1 kg	10 L
5 kg	50 L

Conclusion

AVIPure dsRNA removes dsRNA from mRNA feeds as effectively as RP-HPLC or cellulose/ethanol chromatography.

AVIPure dsRNA is selective for dsRNA with minimal mRNA binding.

Process requires no organic solvents or ion-pairing reagents, only saline buffer.

Prepacked devices in a wide range of sizes will be available.

Want to test AVIPure dsRNA?

Collaborate with us to receive:

- Early access to resin/devices proven with your material
- Updates on commercial launch and supply
- Access to resin for cGMP prior to commercial launch
- Special pricing for first year after commercial launch
- Co-publishing and presentation opportunities

Email nclark@repligen.com for info

References

1) Yasaman Gholamalipour et al. (2018). 3' end additions by T7 RNA polymerase are RNA self-templated, distributive and diverse in character—RNA-Seq analyses. *Nucleic Acids Research*, 46(18), 9253–9263,

2) Kithmie MalagodaPathiranage, Craig T. Martin (2023) A simple approach to improving RNA synthesis: Salt inhibition of RNA rebinding coupled with strengthening promoter binding by a targeted gap in the DNA. *Methods in Enzymology*, Academic Press

3) Baiersdörfer M, et al. (2019) A Facile Method for the Removal of dsRNA Contaminant from In Vitro-Transcribed mRNA. *Mol Ther Nucleic Acids*. 15, 26-35

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