Min-Immune™ Gold dsRNA Removal Across Diverse IVT Synthesis Workflows

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Introduction

The presence of double-stranded RNA (dsRNA) in mRNA preparations is a well-established trigger of innate immune responses primarily through activation of pattern recognition receptors such as Toll-like receptor 3 (TLR3), retinoic acid-inducible gene-I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5). These responses can significantly compromise the efficacy and safety of mRNA-based therapeuticsand vaccines. Efficient removal of dsRNA contaminants is critical for minimizing immunogenicity and ensuring optimal performance in downstream applications.

Conventional dsRNA removal techniques including reverse-phase high-performance liquid chromatography (RP-HPLC), hydroxyapatite chromatography and cellulose-based methods, are often associated with high capital investment, complex workflows and reduced recovery of desired single-stranded RNA (ssRNA). In contrast, CELLSCRIPT™'s Min-Immune™ Gold dsRNA Removal Kit offers a novel, enzymatic approach that is economical, scalable and user-friendly, with a minimal loss in product yield. It enables the reduction of dsRNA to less than 0.005% of the total RNA content.

This study demonstrates the ability of the Min-Immune Gold dsRNA Removal Kit to treat *in vitro* transcribed (IVT) RNA, regardless of the source of the kit used for transcription.

Materials and Methods

To evaluate the effectiveness of the Min-Immune Gold dsRNA Removal Kit, firefly luciferase RNA was synthesized using four popular commercially-available IVT kits from CELLSCRIPT, NEB, Invitrogen, and Promega. Manufacturer supplied instructions were followed for each kit.

Following transcription, RNA samples were processed following the Min-Immune Gold dsRNA Removal Kit instructions. More specifically, kit components were combined with the IVT RNA and incubated at 37°C for 1 hour. Following treatment, RNA was precipitated with NH_4OAc, washed with 70% ethanol, resuspended in RNase-free water and quantified by spectrophotometry.

The extent of dsRNA reduction was assessed by comparing treated and untreated samples using a slot blot immunoassay employing a dsRNA standard curve and dsRNA-specific J2 monoclonal antibody (SCICONS). The standard was loaded in triplicate onto a nitrocellulose membrane with total quantities of dsRNA between 50 and 250 pg. To align expected assay output for both treated and untreated samples within the dynamic range of the assay, untreated samples were loaded with a final quantity of 100 ng per sample replicate and Min-Immune Gold-treated samples were loaded at 1,000 ng per sample replicate. The final quantity of calculated dsRNA accounts for this difference in sample input. Both untreated samples and Min-Immune Gold-treated samples were loaded in triplicates. After blotting, the membrane was air-dried, blocked with a 5% (w/v) skim milk in TBST, incubated overnight with J2 monoclonal anti-dsRNA antibody (SCICONS), and then incubated with Anti-mouse IgG, HRP-linked antibody. Signal detection was performed using enhanced chemiluminescence (ECL) on a Syngene® G:Box imaging system. Sample signal intensities were compared to intensity of the standard curve to quantify dsRNA content. Linear regression analysis was used to quantitate the relative levels of dsRNA before and after treatment.

dsRNA Content Testing

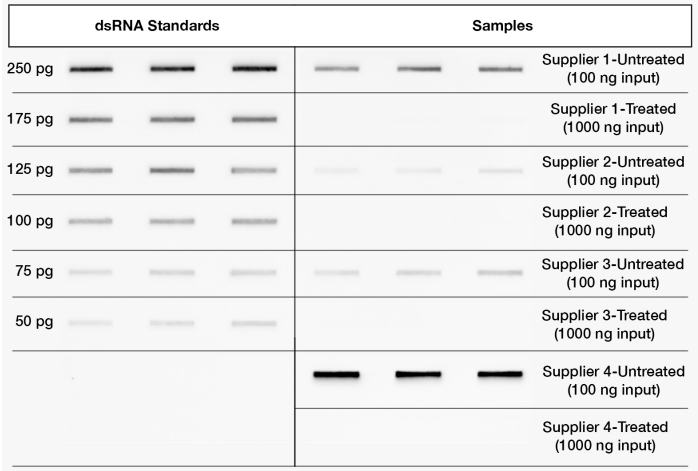

Figure 1. Slot blot image taken on Syngene® G:Box imaging system. The dsRNA standard curve is shown on the left panel. Triplicates of each sample condition can be seen on the right panel.

Table 1. Results from linear regression analysis used to quantify the percentage of dsRNA present in both untreated RNA and Min-Immune Gold-Treated RNA.

Sample	Sample Condition	Percent dsRNA
Supplier 1	Untreated IVT	0.143 %
	Min-Immune Gold Treated	< 0.005 %
Supplier 2	Untreated IVT	0.045 %
	Min-Immune Gold Treated	< 0.005 %
Supplier 3	Untreated IVT	0.063 %
	Min-Immune Gold Treated	< 0.005 %
Supplier 4	Untreated IVT	0.394 %
	Min-Immune Gold Treated	< 0.005 %

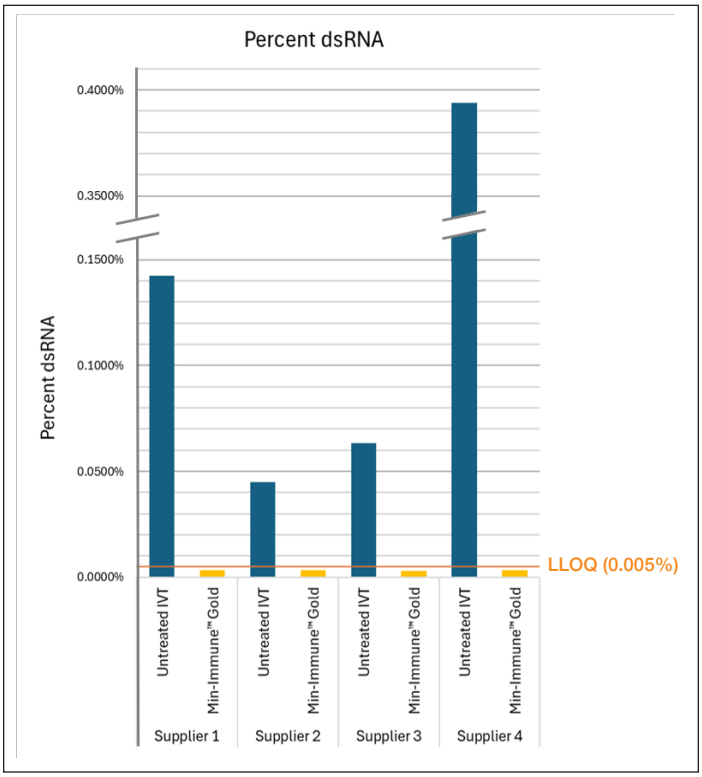

Figure 2. Chart showing percent dsRNA content for IVT RNA produced by each suppliers' kit both before and after Min-Immune Gold dsRNA removal treatment. All Min-Immune Gold-treated samples fell below the lower limit of quantitation (LLOQ) of the assay (0.005%).

Results and Conclusions

Treatment of *in vitro* transcribed firefly luciferase RNA with the Min-Immune Gold dsRNA Removal Kit significantly reduced double-stranded RNA (dsRNA) across all transcription systems tested. These results were reproducible across multiple commercially-available IVT platforms.

The dsRNA standard used for this testing was developed for our validated, in-house assay for quality control testing of full-length mRNAs. The affinity of the J2 monoclonal antibody to a dsRNA standard, and therefore the lower limit of quantitation of an assay, is dependent on both the length of dsRNA regions and the nucleotide composition of the standard. Testing using this in-house method revealed that untreated IVT RNA preparations contained 0.045-0.394% dsRNA content,—nearly a ninefold range—depending on the transcription kit used. In contrast, Min-Immune Gold treated samples consistently exhibited dsRNA levels below the assay's lower limit of quantification (≤0.005%), indicating near-complete removal of detectable dsRNA.

Because dsRNA is a primary driver of innate immune activation, its elimination is essential for maximizing mRNA performance. By substantially reducing these immunostimulatory impurities, the kit supports improved tolerability, more consistent expression, and stronger translational relevance of mRNA constructs. While nucleoside-modified mRNA was not evaluated in this study, the underlying chemistry is fully compatible with modified RNA. The streamlined workflow is also designed for scalability, supporting applications from early-stage research through high-throughput and large-scale mRNA production.