

Rapid quantitative detection of dsRNA impurities in mRNA therapeutics using a novel lateral flow assay



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ABSTRACT

Objective: The rise of mRNA-based therapeutics has revolutionized modern medicine, exemplified by the success of COVID-19 vaccines. These therapies are typically manufactured via in vitro transcription (IVT), a process that inevitably generates double-stranded RNA (dsRNA) as a byproduct. dsRNA contaminants pose serious safety risks due to their potent immunostimulatory properties, which include inducing cytokine release, cellular toxicity, and inhibiting translation of therapeutic mRNA. Current analytical dsRNA detection methods, such as dot blot and ELISA, are limited by insufficient sensitivity, long assay durations, and labor-intensive workflows. This creates a critical bottleneck in process development optimization of mRNA manufacturing.

Methods: To overcome these challenges, we developed a rapid and quantitative lateral flow immunoassay utilizing a dual-antibody system (K1 and J2 mouse anti-dsRNA monoclonal antibodies) for the detection of dsRNA impurities in mRNA IVT reactions. In this assay, 20 μ l of sample is applied to a lateral flow cassette where J2-gold nanoparticle complexes bind to dsRNA targets. These complexes then move via capillary action to a test (T) line coated with K1 dsRNA antibodies, producing a colorimetric signal proportional to dsRNA concentration. Excess J2-gold complexes continue to a control (C) line containing immobilized anti-mouse antibodies, enabling internal validation and signal normalization. Following sample migration, results are captured and analyzed with the user-friendly GoStix™ Plus smartphone app. This software compares the ratio of test-to-control (T/C) band color intensities against a preloaded, lot-specific standard curve and automatically calculates the GoStix Value (GV).

Results: This assay delivers results in 10 min and achieves a quantitation limit of 6.25 ng/ml (0.000625% in a 1 mg/ml IVT sample), using poly(I:C) as a dsRNA standard. It demonstrates high structural specificity for dsRNA over ssRNA and dsDNA, with signal performance influenced by dsRNA length and modification. This method also shows excellent precision, accuracy, and reproducibility across replicate tests.

Conclusion: This user-friendly, rapid, quantitative detection of dsRNA impurities—with minimal hands-on time—is a valuable tool for enhancing product safety, streamlining manufacturing workflows, and supporting research and process development in mRNA production.

1 10-min workflow for dsRNA quantitation using GoStix Plus and smartphone app

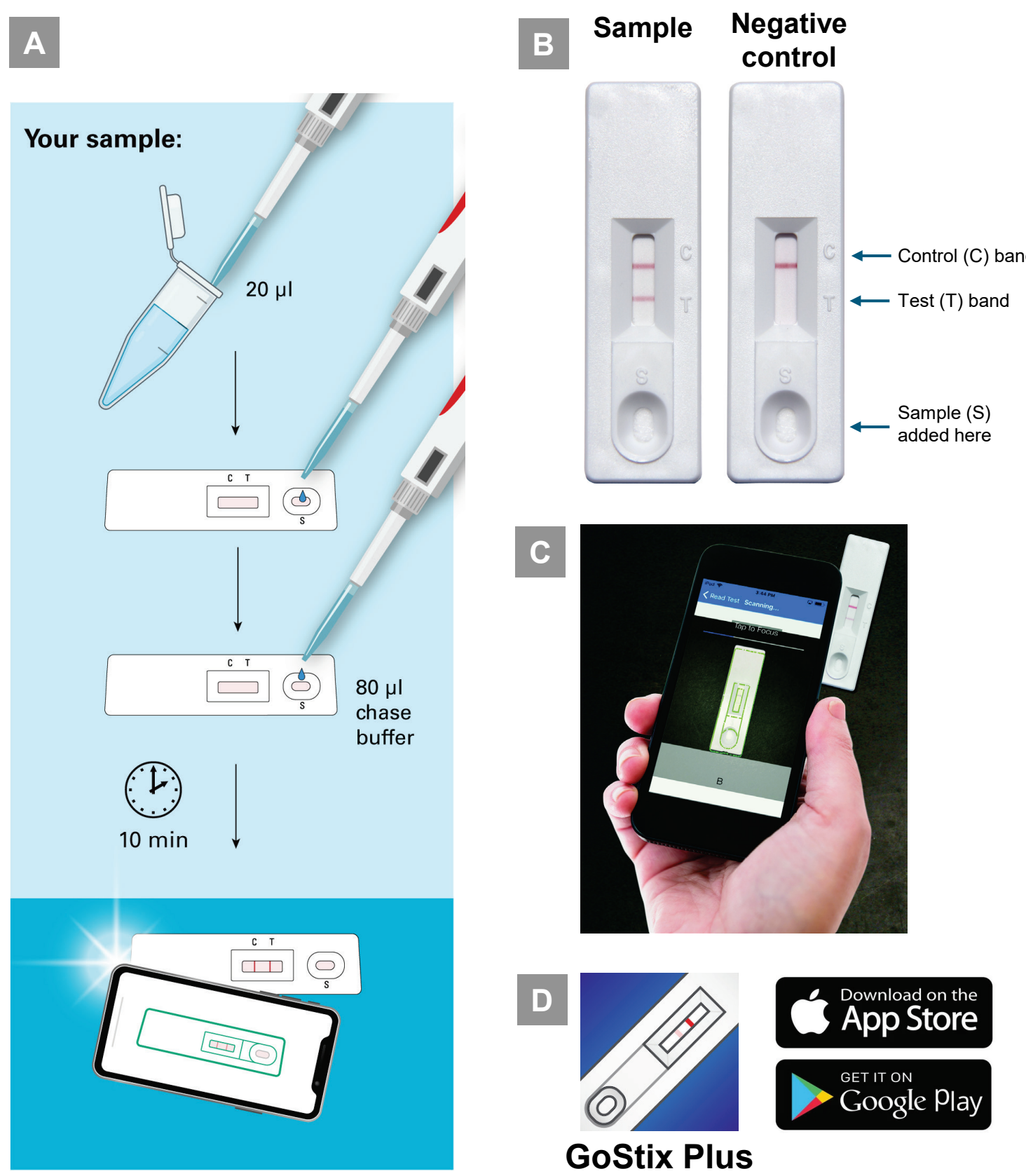


Figure 1. dsRNA titration is fast and easy with the GoStix Plus cassette and app. Panel A. This lateral-flow assay detects dsRNA present in IVT mRNA samples. Simply add 20 μ l of sample, followed by chase buffer, and incubate at room temperature for 10 min. Panel B. Test and control bands develop during the 10-minute incubation time. Panel C. Band intensities are analyzed and quantified using the GoStix Plus smartphone app. Panel D. The GoStix Plus app can be downloaded from the App Store or Google Play.

2 Schematic of dsRNA GoStix Plus

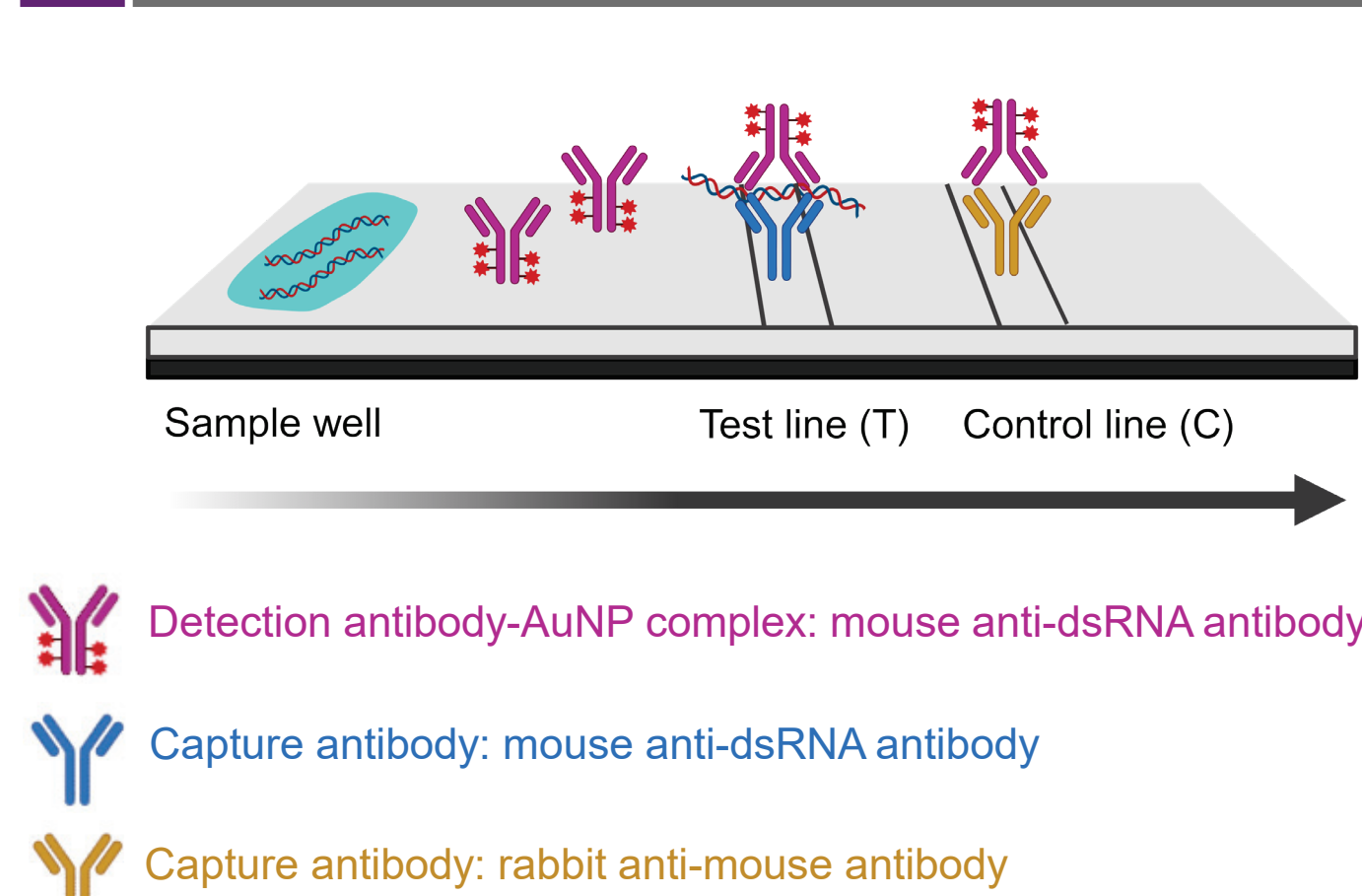


Figure 2. Schematic of dsRNA GoStix Plus test. In this assay, 20 μ l of sample is applied to a GoStix Plus cassette where detection antibody-gold nanoparticle (AuNP) complexes bind to dsRNA targets. These complexes then move via capillary action to a test (T) line coated with capture anti-dsRNA antibodies, producing a colorimetric signal proportional to dsRNA concentration. Excess detection antibody-AuNP complexes continue to a control (C) line containing immobilized capture rabbit anti-mouse antibodies, producing a colorimetric signal, enabling internal validation and signal normalization.

3 Antibody clones used for dsRNA capture and detection in the lateral flow cassettes were selected to ensure the strongest signal

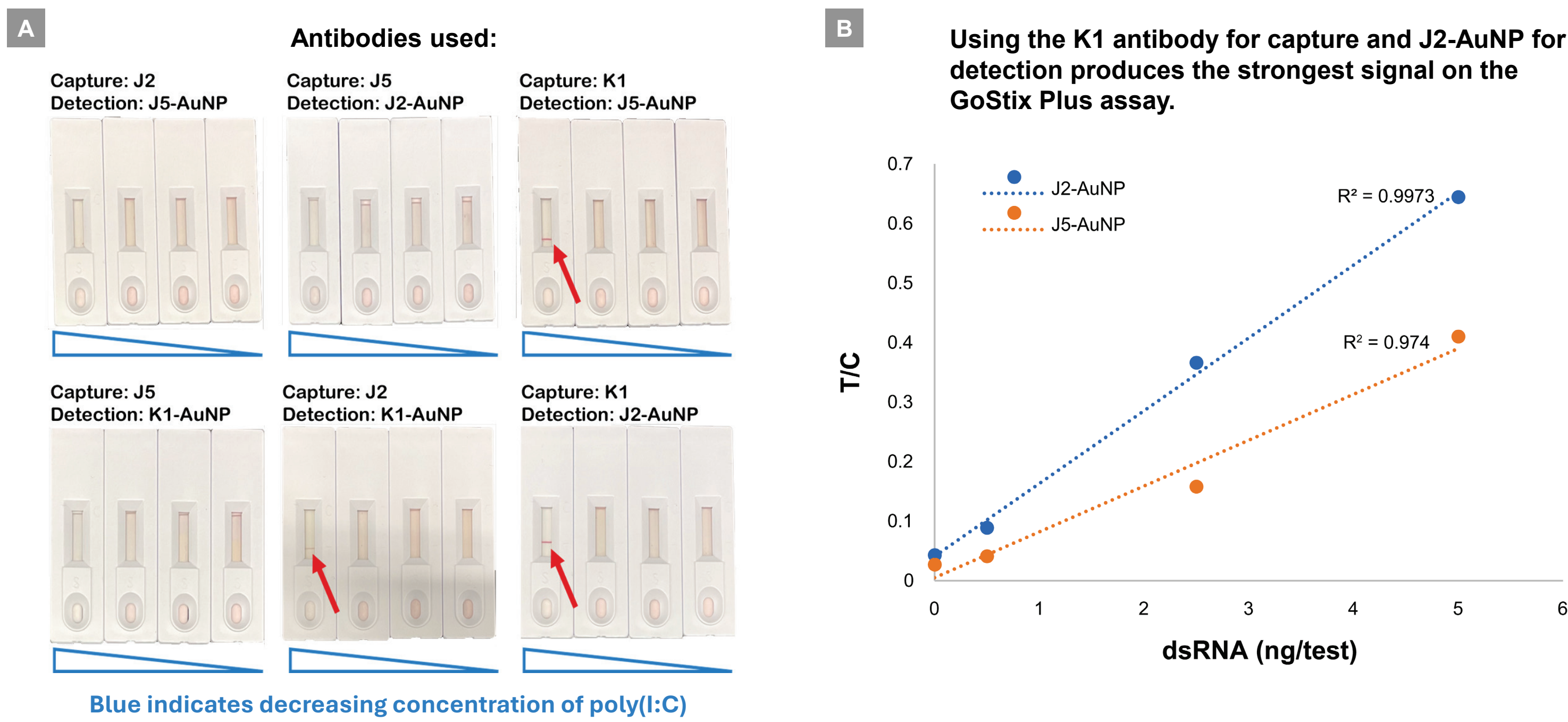


Figure 3. For assay optimization, six different configurations of three anti-dsRNA antibody clones were tested to determine which antibody used for detection and which antibody used for capture would produce the strongest signal on the GoStix Plus cassette. Panel A. J2, J5, and K1 anti-dsRNA antibodies were passively adsorbed onto gold nanoparticles to be used for dsRNA detection. Different possible combinations of the J2, J5, and K1 antibodies were used for capture. pH and concentration were optimized for efficient binding. All six combinations were tested with varying concentrations of poly(I:C) (used as a dsRNA reference). Three combinations produced a detectable signal at the highest concentration of poly(I:C), as indicated with the red arrows. The two antibody combinations with the highest signal were selected for further investigation. Using K1 for capture and either J2-AuNP or J5-AuNP for detection produced titers comparable to the poly(I:C) control, with J2 showing stronger signals than J5.

4 Antibodies showed high specificity for dsRNA over ssRNA

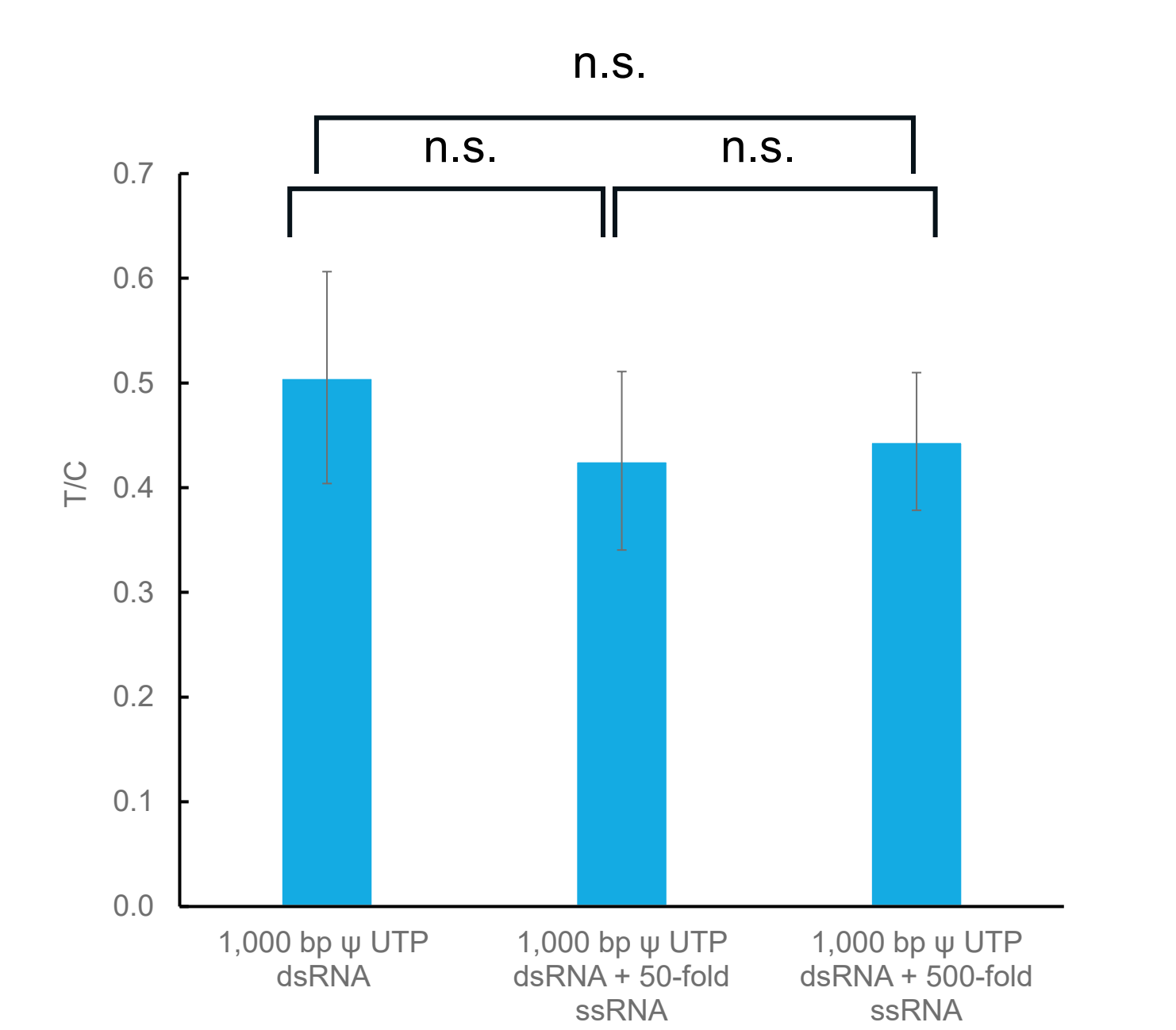


Figure 4. Specificity of the antibody for dsRNA over single-stranded RNA (ssRNA). GoStix T/C values of 1,000 bp ψ UTP dsRNA in the presence of 50-fold or 500-fold excess ssRNA. No significant differences (n.s.) in T/C values were observed among the three groups. Statistical analysis was performed using one-way ANOVA.

5 Establishment of poly(I:C) control curve for dsRNA quantitative detection in the smartphone app



Figure 5. Establishment of control curve. A standard curve was generated using average T/C values from a dilution series of poly(I:C) prepared in chase buffer (Panel A). Samples were run on GoStix Plus cassettes in triplicate, developed for 10 min (Panel B), and analyzed using the GoStix Plus App on both iOS and Android devices. T/C ratios corresponding to each poly(I:C) concentration were plotted to create the standard curve (Panel A). This standard curve is preloaded in the app and does not need to be recreated by the user. When poly(I:C) is used as a reference, the app uses a linearized equation from this curve to automatically quantify dsRNA levels in unknown mRNA samples, with results reported in ng/ml of poly(I:C) equivalents.

6 GoStix Plus measures a similar dose-dependent response as ELISA

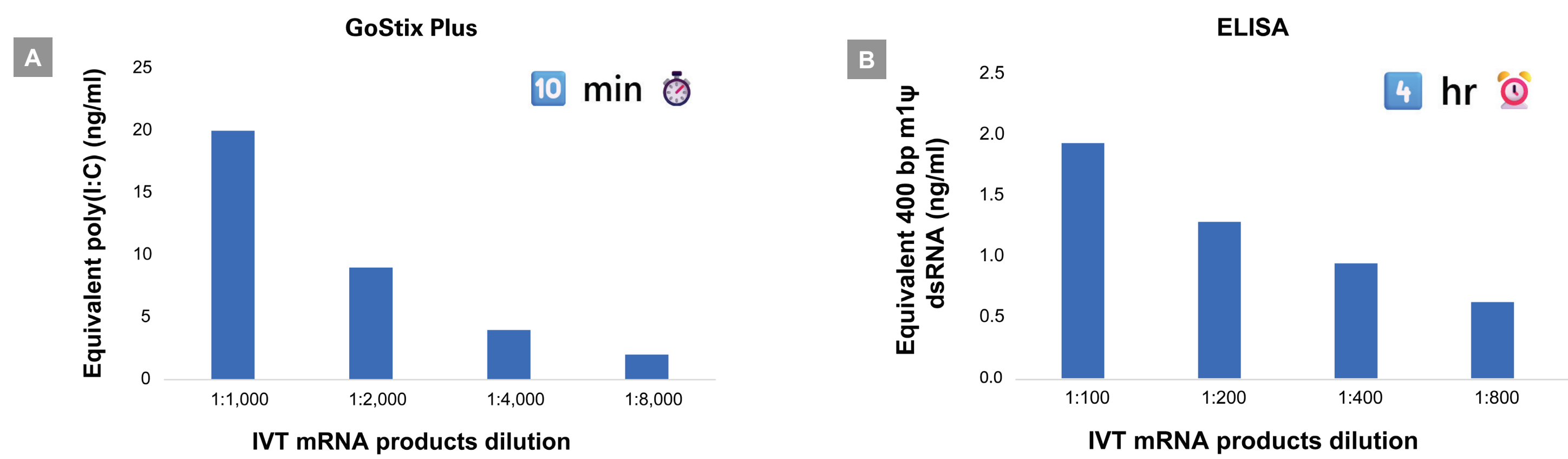


Figure 6. Detection of dsRNA in IVT mRNA using GoStix Plus and J2-based ELISA. IVT mRNA was synthesized using the Takara IVTpro™ T7 mRNA Synthesis Kit (Takara Bio, Cat. # 6144) with the FLuc positive control template provided in the kit. dsRNA levels were measured using (Panel A) GoStix Plus and (Panel B) a J2 antibody-based dsRNA ELISA (GenScript, Cat. # L01020). Both assays demonstrated similar dose-dependent responses. Notably, the GoStix Plus assay was completed in 10 minutes, whereas the ELISA required 4 hours.

7 Create a standard curve specific to the length and modifications of your dsRNA with less hands-on time using GoStix Plus

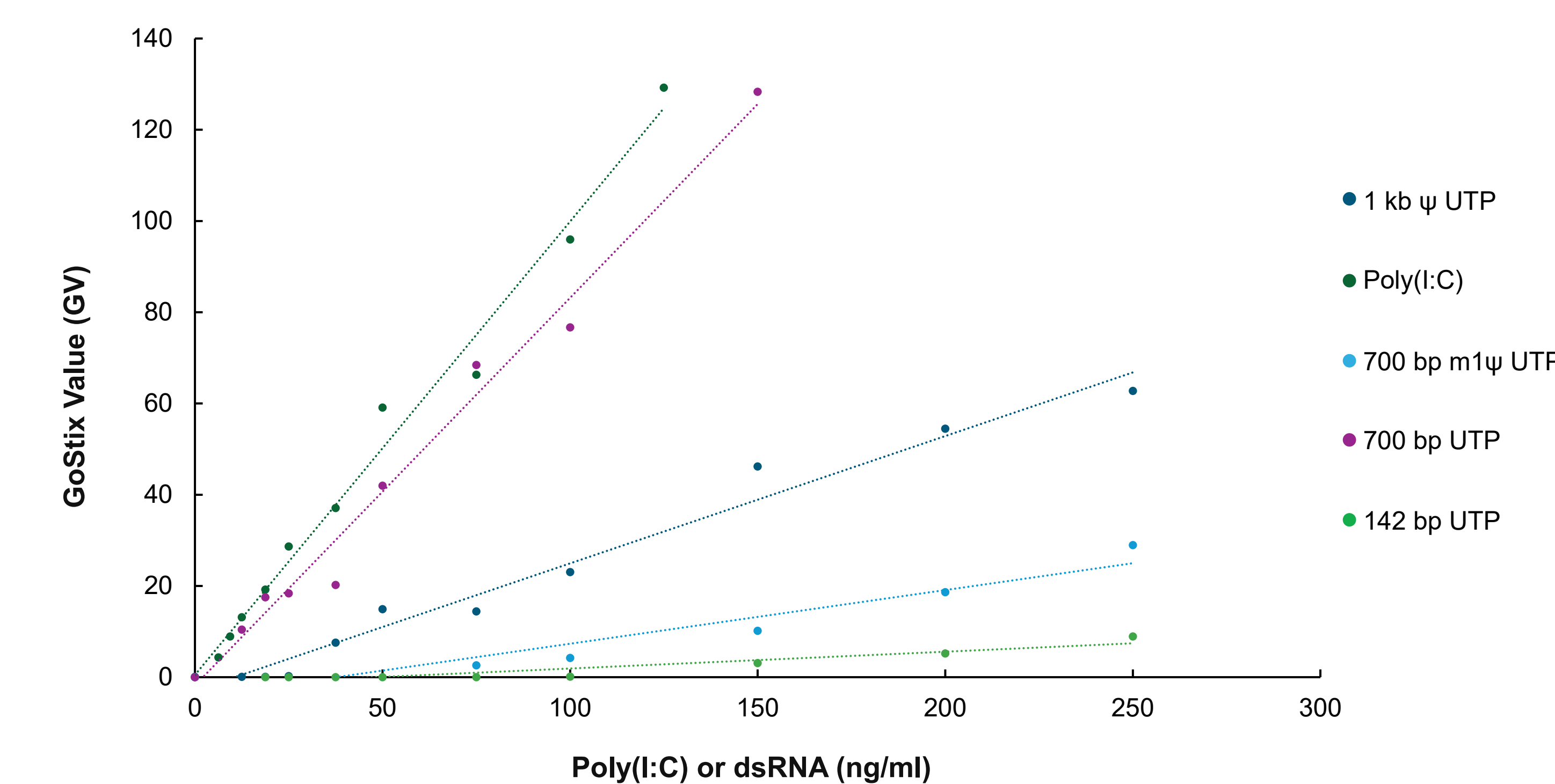


Figure 7. dsRNA length and modifications impact GoStix Value quantitation, similar to ELISA. dsRNA GoStix Plus was used to evaluate the effect of dsRNA length (142 bp, 700 bp, and 1 kbp) and chemical modifications (e.g., m1 ψ or ψ) on test line signal intensity. 20 μ l of each sample, containing varying concentrations of different dsRNA species in STE buffer, were applied to GoStix Plus cassettes, followed by 80 μ l of chase buffer. After a 10-min incubation, the cassettes were scanned, and GoStix Values were recorded and analyzed. Results indicate that both dsRNA length and chemical modifications influence the GoStix signal for dsRNA quantitation.

8 dsRNA GoStix Plus specifications

Table 1: technical specifications

Detection range	6.25–125 ng/ml poly(I:C)
R ²	>0.970
CV	<20%

CONCLUSIONS

- Streamlined workflow with a 10-minute protocol**—reduces hands-on time and operator-related variability while maintaining accuracy
- Reliable and accurate**—delivers strong linear correlation of quantitation values and target concentration, across poly(I:C) and dsRNA standard dilutions, ensuring consistent and dependable results
- Comparable results to ELISA, in less time**—achieves detection that closely correlates with ELISA results, offering a rapid and robust alternative for routine dsRNA quantification



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