Check your RNA quality with excellent speed and accuracy on Lunatic

RNA is silver, pure RNA is gold

Why do you need pure RNA?

Checking the purity of RNA from tissue, cell/bacterial-cultures, or *in vitro* transcribed mRNA is crucial for both therapeutic and analytical applications, from creating RNA-LNPs all the way to genetic expression assays. For instance, during sensitive applications like qRT-PCR, both reverse transcribed mRNA as well as the contaminating genomic or complementary DNA can serve as templates for the subsequent steps. Hence, the carryover of DNA can potentially produce non-specific results and throw a bunch of noise into a PCR or RNA-seq experiment.

Why can obtaining pure RNA be so tricky?

Acid guanidinium thiocyanate-phenol-chloroform (AGPC)[1] and TRIzol™ are popular RNA solution-extraction methods. In both of these, RNA is phase-separated from DNA and proteins into district layers by means of aqueous and organic solvents. The bottom organic phase contains proteins, the middle interphase contains DNA, and the top aqueous phase contains RNA. When pipetting off the top phase containing RNA, contamination with DNA or protein from other phases is a major risk. Contamination with proteins and/or solvents potentially inhibits later DNase treatment, decreases purity and lowers the amount of retrieved RNA. Also, as the last steps of RNA isolation include top phase centrifugation and washing the RNA pellet, improper washing of pelleted RNA could leave behind AGPC and TRIzol™ components.

How to assess RNA purity?

The traditional way to evaluate quality and quantity of extracted RNA is a UV/Vis absorbance measurement at 260 and 280 nm. An A260/A280 absorbance ratio between 1.8 and 2.0 indicates pure RNA, but by itself it will not say anything about what contaminant you've got in the sample and will leave you wondering about the actual RNA quantity. It is especially tricky to evaluate the true concentration of RNA contaminated with DNA, since the two molecules have similar absorbance.

A

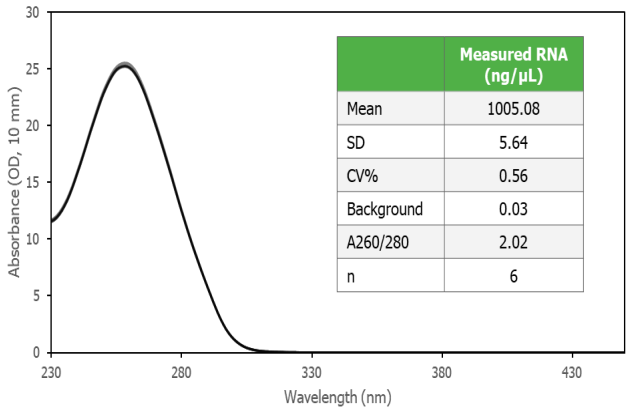
B

Figure 1: Lunatic is the next-generation UV/Vis system for nucleic acid (and protein) quantification (**A**). High Lunatic plates have 96 individual microfluidic circuits that only use 2 µL per sample and read up to 96 samples at a time (**B**).

Upgrade your RNA quality control with Lunatic

Pure RNA is no sweat

Lunatic (Figure 1) makes RNA quantification easy with a wide dynamic range that can measure concentrations from 1.2 to 11,000 ng/µL RNA (0.03-275 OD). UV/Vis absorbance is read from



	Measured RNA (ng/µL)
Mean	1005.08
SD	5.64
CV%	0.56
Background	0.03
A260/280	2.02
n	6

Figure 2: Overlaying absorbance spectra of calf liver RNA. The inset table shows the average concentration, standard deviation (SD), coefficient of variation (CV%), background absorbance at 260 nm and A260/A280 ratio of 6 replicates (n).

just 2 µL of sample and up to 96 samples can be measured in only 5 minutes (Lunatic plate) or 10 minutes (High Lunatic plate).

To demonstrate the accuracy and precision of Lunatic, purified calf liver RNA at approximately 1,000 ng/µL was measured with the "RNA (Turbidity)" application.

The results for six replicates of the pure RNA sample show overlaying baseline and turbidity-corrected UV/Vis spectra (Figure 2). These results show off Lunatic's high degree of precision across replicates, along with the low coefficient of variation (CV%) and standard deviation (SD). Lunatic delivers extreme precision, without the hassle of using dyes, additional reagents or preparing standard curves.

Don't get fooled by messy RNA

Lunatic's Unmix applications see through RNA contaminants to quantify RNA concentration by separating the absorbance contribution from RNA and co-absorbing impurities. The "RNA (Any source)" application (Figure 3A) is specifically designed to deconvolute RNA absorbance from DNA, protein and common extraction buffers, giving you an RNA concentration to figure out if you're good to go, or if more purification is needed (Figure 3B and C).

Handle a broad range of RNA/DNA ratios

When DNA is the contaminant to your RNA sample, Lunatic can separate the signals from RNA and

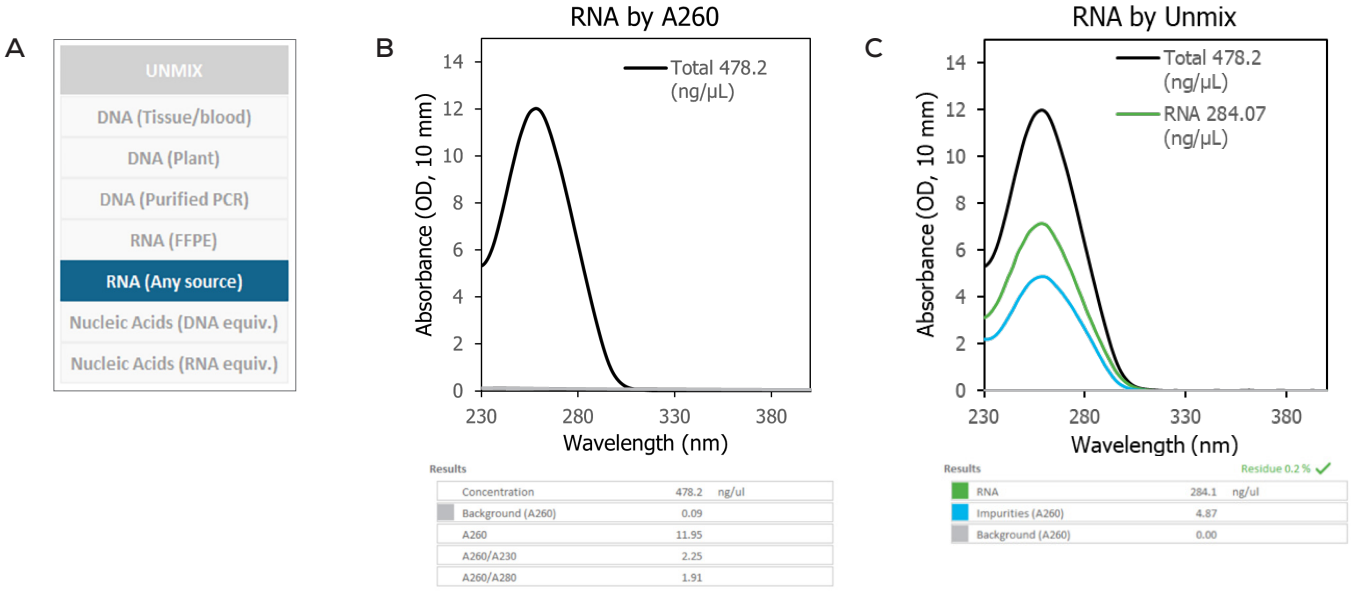


Figure 3: Unmix "RNA (Any source)" quantifies concentration of RNA derived from various materials. Unmix algorithms subtract sample background and various impurities to deconvolute the absorbance spectrum and concentration of RNA (**A**). A traditional measurement of contaminated RNA at A260 does not provide full information about potential sample contamination apart from A230/A260 and A260/A280 ratios (**B**). These ratios indicate sample purity in terms of protein contamination but they are unable to distinguish nucleic acids. For RNA samples where contamination is a risk the "RNA (Any source)" application sees through RNA contamination and delivers an RNA concentration (**C**). Lines/squares denote: black - UV/Vis spectrum of total nucleic acids, green - RNA concentration, turquoise – impurities, gray – background turbidity. Tables at the bottom (**B, C**) are on-screen reports.

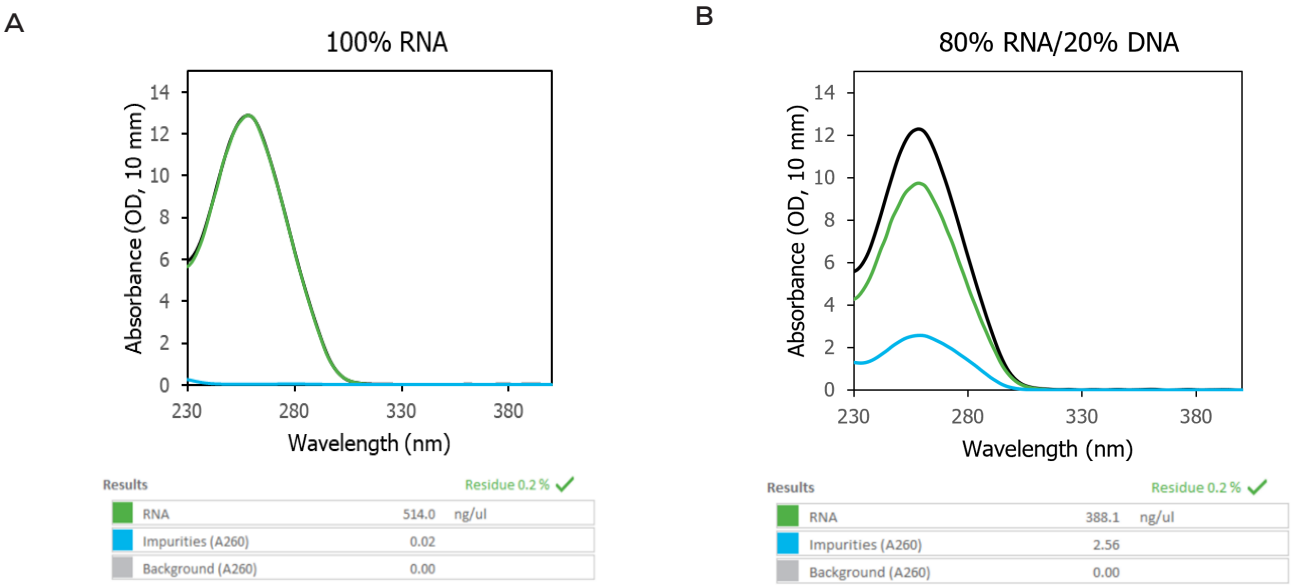


Figure 4: Unmix "RNA (Any source)" application reports DNA contamination. Absorbance spectra and results from pure (**A**) and DNA-contaminated (**B**) samples. Without DNA, total measured nucleic acid and RNA concentration overlap and Lunatic reports trace levels of impurities (**A**). The addition of DNA increases the impurities absorbance value and decreases measured RNA concentration (**B**). Lines/squares denote: same as **Figure 3**.

DNA for a broad range of RNA/DNA ratios – and still report the actual RNA concentrations.

Purified RNA was contaminated with pure calf thymus DNA ranging from as little as 5% all the way up to 40% DNA, while maintaining the total nucleic acid concentration at approximately 500 ng/µL. The "RNA (Any source)" app reports negligible amounts of impurities in the pure RNA sample (Figure 4A), while the 20% DNA samples show increased impurities values and correctly decreased RNA concentration (Figure 4B).

The measured RNA concentrations in different RNA/DNA ratio mixes show a strong linearity as DNA contamination increases. A linear regression of the measured versus target concentrations shows an R^2 of 0.9925 (Figure 5). These results demonstrate that the Unmix algorithm successfully detects contaminant DNA and reports an RNA concentration even in the most contaminated samples.

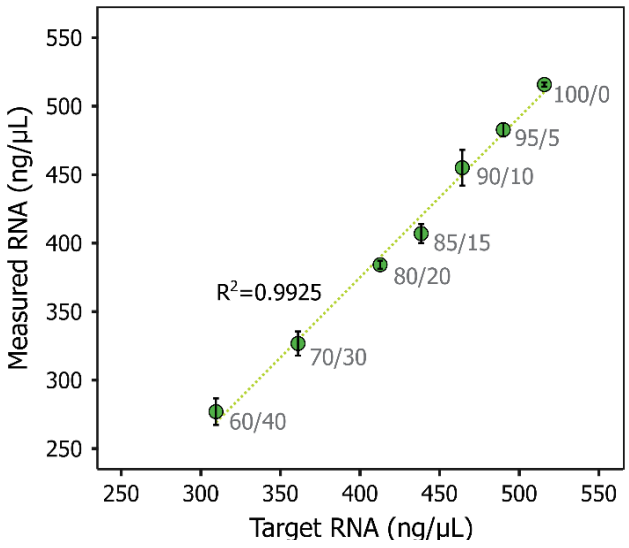
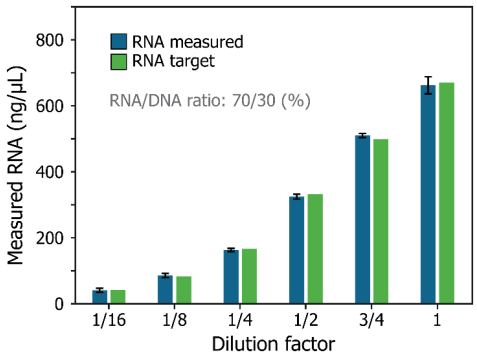


Figure 5: The "RNA (Any source)" app measures RNA concentration at various ratios of RNA and DNA and across a broad range of DNA contamination. Dark green dots represent the measured averages vs. target concentrations of RNA and dashed line shows the linear regression. Inset numbers (gray) represent RNA/DNA ratios (%), adjusted to maintain total nucleic acids concentration at approximately 500 ng/µL. Error bars denote the standard deviation, n= between 9 and 12.

Tackle lots or a little bit of RNA

RNA purification can yield a lot or a little bit of RNA, so to test a wide range of concentrations, RNA and DNA were mixed at a 70/30 ratio, diluted

A



B

Dilution factor	Measured RNA (ng/µL)	Measured nucleic acids (ng/µL)	Percent RNA
1	662.1	956.9	69%
3/4	509.8	713.4	71%
1/2	324.5	474.3	68%
1/4	162.8	237.4	69%
1/8	85.7	119.0	72%
1/16	40.8	59.2	69%

Figure 6: RNA is deconvoluted from contaminant DNA at diverse concentrations. Measured averages of RNA (blue) spiked with DNA in a 70/30 ratio vs. target RNA (green) concentrations (**A**). Averages of measured RNA and total nucleic acid concentration, and calculated RNA percentage (**B**). Values are within 70±2% compared to 70% target. Error bars denote the standard deviation. n= between 12 and 14.

from 1,000 to 62.5 ng/µL (dilution factor 1 to 1/16) of total nucleic acid and measured on Lunatic.

The "RNA (Any source)" application has no problem across this concentration range. The measured RNA consistently makes up 70±2% of the total measured nucleic acids concentration, compared to the 70% target (Figure 6A and B). In other words, the measured RNA is spot-on the expected level throughout all tested concentrations. Unmix gives Lunatic the power to accurately report the actual RNA amount in a DNA-contaminated sample through a broad range of nucleic acid concentrations.

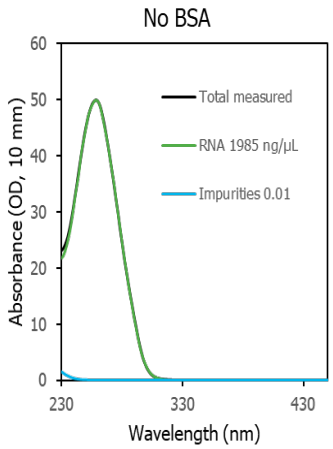
Proteins are no problem

Protein contamination is also common in RNA extraction. Since proteins absorb strongly at 280 nm, their presence in an RNA sample gets in the way of RNA quantification. Lunatic's Unmix deconvolutes nucleic acids and protein spectra to correctly assess RNA quality and concentration.

To test this out, purified RNA at approximately 2,000 ng/µL was spiked with Bovine Serum Albumin (BSA) at 10% and 50% v/v.

The results show that RNA concentration decreases as the amount of added BSA increases (Figure 7). These results show that with Lunatic you don't have to

A



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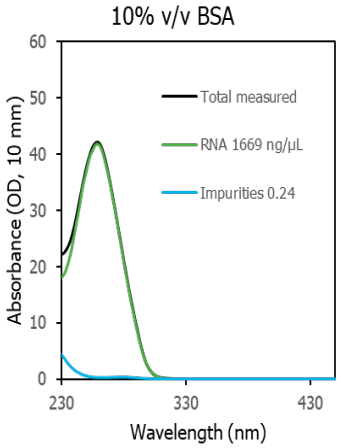

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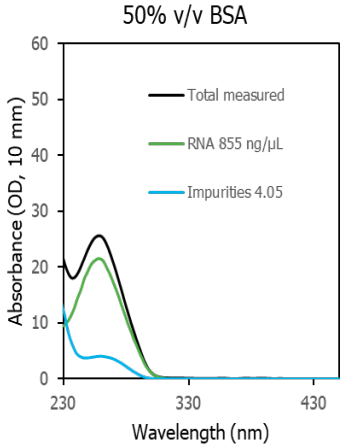

Figure 7: Unmix deconvolutes protein signal in RNA solutions. Absorbance spectra and results from the Unmix "RNA (Any source)" application. Absorbance spectrum of pure RNA (**A**) and RNA spiked with v/v 10% (**B**) and 50% (**C**) Bovine serum albumin (BSA). Addition of BSA lowers the measured RNA concentration as the RNA is diluted by the spike-in. Unmix algorithm spots BSA and provides an actual RNA concentration. Lines/squares denote: same as **Figure 3**. n= between 3 and 4.

worry that contaminating proteins will go undetected. By checking out the impurities absorbance spectrum you can see when proteins are present and still get an accurate RNA measurement thanks to Unmix.

Conclusion

Pure RNA is the critical starting point for many rapidly growing therapeutic and analytical applications, but RNA often suffers from unfavorable contamination even after purification. Lunatic's "RNA (Any source)" application can see through contamination from DNA and protein to measure RNA concentration and keep an eye on the impurities present. There's no need to prep dyes, run a standard curve, or risk introducing pipetting errors from sample dilutions. Simply load your samples, press the start button and measure dirty or pure samples with high-throughput and precision. That makes Lunatic a game-changer for genomics.

Methods

RNA from calf liver (Sigma-Aldrich, R7250) was diluted in RNA storage solution (Ambion; AM7001) and further filtered through 100 kDa centrifugal filter (Merck; UFC210024) at 4,000 rpms for 15 min. Purified solution was diluted with RNA storage solution to desired concentration and stored at -20 °C. Calf thymus DNA (Invitrogen; 15633-019) was diluted with deionized water (VWR; 23595.294) to desired concentration and stored at -20 °C. Bovine serum albumin solution (Sigma-Aldrich; A7284) was freshly diluted in Phosphate Buffered Saline (Life Technologies 10010-015).

All measurements were performed using High Lunatic plates. Samples were handled on ice at all times.

In Figure 5 and Figure 6 outliers were identified and removed *via* interquartile range (IQR) method. Data in Figure 5 and Figure 6 were plotted RStudio (version 2022.02.1) with R (version 4.1.3).

References

1. Chomczynski, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159 (1987).

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