RNase A Contamination: Unpredictable and Catastrophic

Prevent Contamination in your Lab with High Irradiance UV LED

A Phoseon Technology White Paper

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Ribonuclease (RNase) Contamination: Unpredictable and Catastrophic

RNases are an ongoing problem for experiments requiring full length RNA. Even trace amounts of RNase have a big impact on RNA sequencing.

The single most important aspect of RNA protocols is isolating and maintaining full length, un-degraded RNA for analysis or use as a reaction substrate. Whether preparing total RNA libraries for NGS or looking at individual RNAs (iCLIP), un-degraded RNA is pivotal. Hindering these processes is RNase.

Degradation by nucleases such as RNase is a recurring laboratory handling issue which requires diverse cleaning methods that are both time-consuming and costly. Repeated cleaning not only leaves residues, another source of contamination, but can also affect the integrity of equipment (corroding metal and degrading plastics). It is also not always clear when these cleaning methods should be implemented.

Furthermore, repeated exposure to cleaning solutions or soaking may corrode metal or degrade plastic surfaces. How clean is clean enough? Clean enough occurs when you don't need to repeat lengthy protocols because of degraded RNA. Even trace amounts of RNase have a big impact on RNA sequencing, due to its catalytic action. Not clean leads to time and money loss.

This white paper makes the case for why UV LED technology deserves serious consideration by RNA sequencing labs for controlling ribonuclease. It describes Phoseon's findings related to LED light engines for the inactivation of RNases in a laboratory setting.

Quantification of Ambient RNase A Contamination

RNA Institutional knowledge says the level of contamination is directly proportional to time. Thus one could wait a specified amount of time to clean surfaces or equipment. However, this approach does not address the seemingly random contamination that often plagues RNA-work, a particular concern when it comes to microplates.

The more time microplates spend removed from their sterile environment the greater the contamination. Unsurprisingly, we found that a microplate exposed to a business cubicle environment for 1.5 months showed higher levels of contamination overall then one exposed for just five minutes (figure 1A-B). But contamination is not only proportional to total time of exposure. It also occurs sporadically, resulting in unpredictable variations in contamination levels between individual wells of a microplate.

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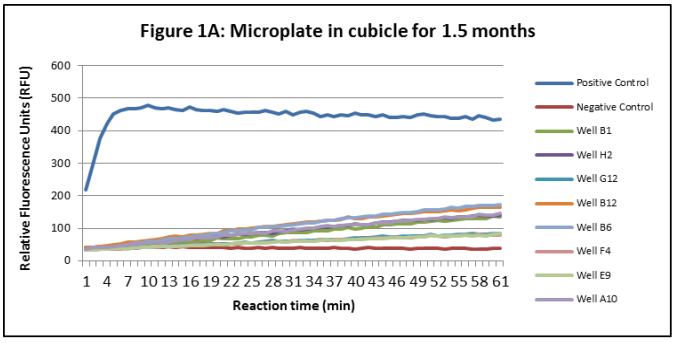

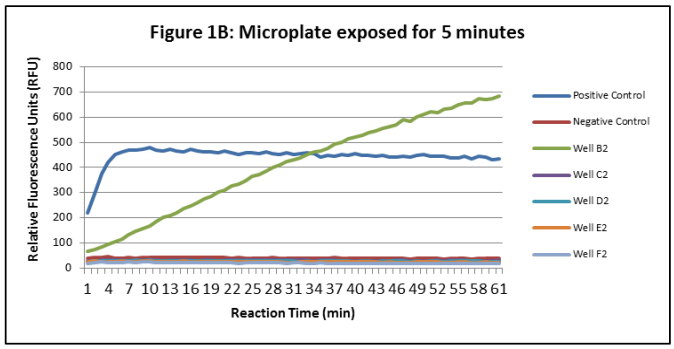

Figure 1: Long-term vs short-term ambient contamination exposure
A flat-bottomed, black opaque microplate was exposed to an office cubicle environment for 1.5 months (a) and a second microplate was exposed to an office lunch-room environment for 5 minutes (b). Sample wells were suspended and fluoroimetrically assayed for RNase activity (RNase Alert IDT, Gemini XPS).

In our studies of RNase A inactivation with a high intensity UV LED scanning array, we found the burden RNase contamination can be significantly reduced.

The microplate exposed to a lunchroom environment for five minutes showed one well with significant levels of contamination above the negative control while all other wells showed no significant contamination (figure 1B). This one well represents instantaneous contamination irrespective of the total time of exposure. Implying that simply limiting exposure to environmental RNase does not ensure your microplate is free of contamination. Up to this point laboratories doing RNA work have been forced to bear the weight of this random ambient contamination in their microplates, repeating lengthy protocols while hoping the contamination is not also repeated. In our studies of RNase A inactivation with a high intensity UV LED scanning array, we found the burden RNase contamination can be significantly reduced.

Working from previous knowledge that 275nm and 365nm sources work synergistically to speed RNase A inactivation (1), we used these wavelengths on Greiner Bio-One flat-bottomed black opaque microplates. Sample wells were first treated with 0.01u/mL of RNase A. We then used 1.4 W/cm ^{2} of 275nm light and 700 mW/cm ^{2} of 365nm light (figure 2C) on a scanning array to decontaminate the doped wells. Once efficacy was confirmed on Greiner plates, we began testing microplates of other materials, colors, and well shapes.

Each plate was first melt-tested to determine the intensity of UV light it could withstand. Some plate materials proved to be incompatible with the high-intensity UV at RNase inactivation wavelengths (figure 2B).

Figure 2A: Tapered Wells

Plate material	Color	Wavelength and power
Polypropylene	White	275nm - 1.0 W/cm² 375nm - 900 mW/cm²
Polystyrene	White	275nm - 1.0 W/cm² 375nm - 900 mW/cm²
PET	White	275nm - 1.0 W/cm² 375nm - 900 mW/cm²
	Black	275nm - 1.0 W/cm² 375nm - 900 mW/cm²
Polycarbonate/ polypropylene	Clear	275nm - 1.0 W/cm² 375nm - 900 mW/cm²

Figure 2B: Round-Bottomed Wells

Plate material	Color	Wavelength and power
Polypropylene	White	275nm - 1.2 W/cm² 375nm - 900 mW/cm²
	Black	275nm - 1.2 W/cm² 375nm - 900 mW/cm²
Polystyrene	White	275nm - 1.4 W/cm²
	Black	275nm - 1.4 W/cm²
Polyvinyl Chloride (PVC)	Clear	*plate was deformed when exposed to any amount of 275 of 375 light.

Figure 2C: Flat-Bottomed Wells

Plate material	Color	Wavelength and power
Polypropylene	Black	275nm - 1.4 W/cm² 375nm - 700 mW/cm²

Figure 2: Inactivation of RNaseA on various microplate types
Microplates with different well shapes, material type and color were tested for the lowest intensity of 275nm alone or 275nm and 365nm light necessary to achieve total inactivation of 0.01u/mL of RNaseA contamination. Light source was a scanning UV LED array with each scan lasting under five minutes.
A: microplates with tapered wells
B: microplates with round-bottomed wells
C: microplate with flat-bottomed wells

We then determined the minimum intensity of light necessary to successfully inactivate RNase A in each plate type. Figure 2 outlines the various kinds of plates that were tested and what levels of UV were necessary.

With a high-intensity, scanning UV LED array we effectively decontaminated the most common microplate types in less than five minutes. This protocol does not leave any residue as chemicals and sprays often do. Furthermore, by implementing this protocol prior to every run, the integrity of your RNA library or sequence is secure.

With a high-intensity, scanning UV LED array we effectively decontaminated the most common microplate types in less than five minutes.

KeyPro™ KP100 Decontamination System

High-intensity LED Light Engines Effectively Inactivate RNase A

High-intensity UV light has been shown to rapidly, effectively, irreversibly inactivate RNase.

Working with RNA can be intimidating. Environmental RNase contamination sources include microbial contamination from room air as well as RNases from human skin, hair, or saliva. RNase inactivation methods range from DEPC treatment followed by autoclaving to more involved methods such as: chemical decontamination of surfaces, baking glassware, rinsing equipment in RNase-free water after chemical treatment, and frequent glove changes - all while continually using freshly opened disposables. Such cleaning methods can be costly in terms of money but more importantly they are time consuming, slowing research throughput and likely leading to erroneous results. Now there is a better solution.

Phoseon Technology is the first to develop a UV LED system that surpasses 2.5 W/cm^2, significantly higher than the levels reached by other technologies in the market. This high-intensity UV light has been shown to rapidly, effectively, irreversibly inactivate RNase. This milestone development provides scientists, researchers and equipment manufacturers the capability to rapidly and reliably control RNase contamination.

RNA Protocols that benefit from UV inactivation of RNase:

1) *Ultra-low input and Single-cell RNA sequencing*
2) *Ribosome profiling*
3) *RNA Exome Capture sequencing*
4) *Targeted RNA sequencing*
5) *Small RNA sequencing*
6) *Total RNA sequencing*
7) *mRNA sequencing*
8) *CRAC (Crosslinking And cDNA analysis)*
9) *iCLIP (individual-nucleotide resolution Cross-Linking and ImmunoPrecipitation)*
10) *NGS of RNAs*

Conclusion

- Specific wavelengths, and wavelength combinations, of UV LED irreversibly inactivate RNase A when used at a sufficiently high irradiance.
- The high irradiance necessary for RNase A inactivation is made possible by Phoseon's SLM technology.
- Wavelengths of 275 nm and 365 nm interact synergistically resulting in faster inactivation, at lower irradiances, than is achievable with either wavelength alone.
- UV LED inactivation of RNase A is much faster than conventional methods and does not leave any chemical residue on surfaces.

In short, RNases are an on-going problem for experiments requiring full length RNA. Application of UV LED technology can benefit researchers through improved reliability of starting materials, shorter time required for preparation and inactivation of RNases, all while protecting valuable RNA samples from degradation and chemical contamination.

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About Phoseon Technology

The world leader since 2002, Phoseon Technology pioneered the use of LED technology for Life Science and Industrial Curing applications. Phoseon delivers innovative, highly engineered, patented LED solutions. The company is focused 100% on LED technology and provides worldwide support.

Contacts

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