Phoseon™ TECHNOLOGY

Controlling Ribonuclease (RNase) with High Irradiance UV LED Light Engines

Breakthrough UV-C Performance Enables Better Control for Lab Managers

A Phoseon Technology White Paper

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Why Ribonuclease (RNase) Contamination is a Problem for RNA Sequencing Labs

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

In a lab environment, 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. Hindering this process is the presence of RNase. Whether preparing total RNA libraries for Next Generation Sequencing (NGS) or looking at individual RNAs (iCLIP), degradation by RNases is a recurring laboratory handling issue requiring diverse cleaning methods.

Once a package of disposables is opened, the contents can become contaminated and no longer suitable for RNA work. Pipettes left out on the bench can accumulate dust and microbial contamination from the room air and need frequent re-cleaning. Cleaning surfaces and equipment with sprays and rinses can leave chemical residues, an additional type of contamination, which may interfere with downstream biochemical reactions.

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 in a laboratory setting. It describes Phoseon's findings related to LED light engines for the inactivation of RNases in a laboratory setting.

Using Multiple Wavelength UV LEDs to Control RNase

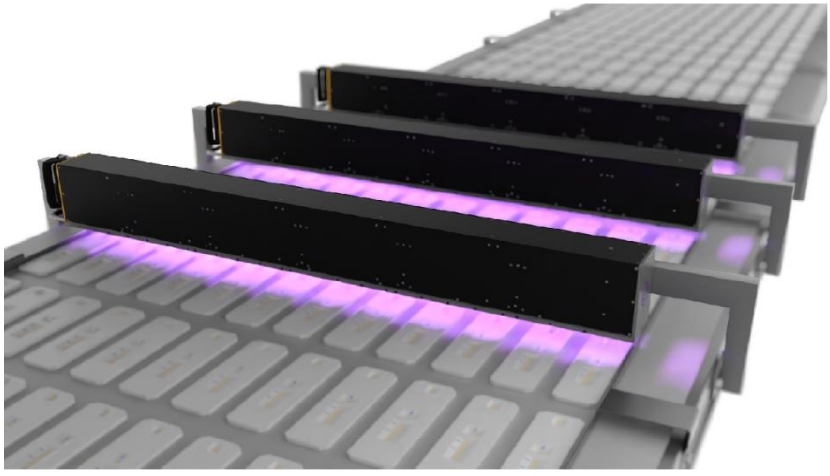
Phoseon's patented Semiconductor Light Matrix (SLM™) technology offers unmatched levels of deep UV irradiance, which enable significant process improvements, including faster analysis and operations, and increased capabilities for disinfection and decontamination applications that require low wavelengths.

UV output from LED systems remains consistent over the long life of the device. That means tighter process control, less downtime, greater lab utilization and overall better and more consistent results.

High irradiance UV-C LED when combined with appropriate wavelengths, targets specific chemical bonds and molecular interactions in DNA, RNA and proteins as well as within microorganisms and biomolecules. This allows shorter inactivation times while improving overall efficacy of the disinfection. The high absolute irradiance of these new solutions enable high-throughput processes in pharmaceutical, sequencing, air handling and manufacturing facilities.

High-irradiance deep UV is opening up disinfection doors.

Offering rapid, consistent, economical inactivation for the lab.



Example of ultraviolet light for disinfection of laboratory surfaces and clinical instruments

UV LEDs Offer New Method for Controlling RNase Contamination

High Intensity, UV decontamination of RNase enables researchers to save significant time and money while ensuring consistent, accurate results.

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 5.0 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*

Irreversible Inactivation of RNases on Surfaces Now Possible with UV LED

RNases, specifically RNase A, are difficult to irreversibly inactivate in the absence of long-term heat treatment or harsh chemicals. Such methods may be incompatible with common laboratory materials or complicate subsequent biochemical reactions. Fast, complete, and irreversible inactivation of RNase A with mercury arc lamp sources have been difficult to achieve due to low power output at targeted wavelengths and the need to filter harmful wavelengths that do not contribute to the inactivation. Enter the UV LED solution.

We report here the use of high irradiance UV LED light engines for enzyme inactivation. Results show that both irradiance (intensity) and radiant fluence (dose) contribute to rapid inactivation of the RNase A enzyme. UV light at 275 nm is thought to act on RNase A via an effect on the aromatic amino acids proximal to disulfide bonds. The 365 nm wavelength is targeted to the lysine side chain with the intent to destabilize the RNase A reaction pocket. These two wavelengths interact synergistically to inactivate RNase A. We conclude that high-intensity UV LED irradiation represents a novel, fast and convenient irreversible inactivation method for RNases on surfaces.

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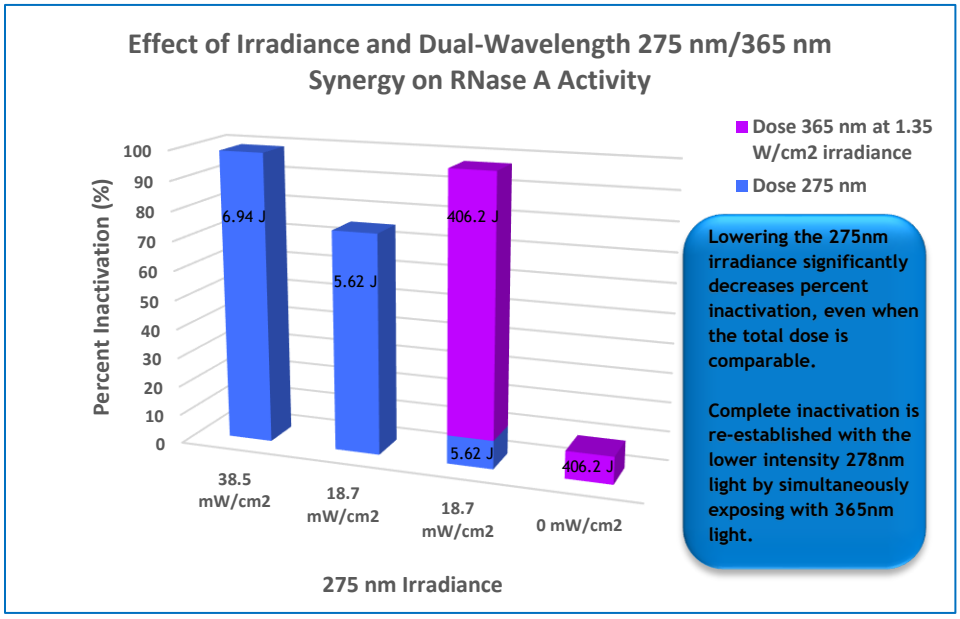


Inactivation of RNases on Microplates

UV-LEDs can lower the burdeon of RNase contamination on a variety of microplates, including those commonly used for PCR, cell-culture and more.

RNase-free microplates are a must for various labs. This includes those doing nucleic acid assays, fluorescence assays that require RNase A incubation, and more. At issue for these labs is the fact that once a new package of plates is opened, even if it is left in the RNase-free hood, one can no longer ensure its sterility. With this in mind we tested as-needed disinfection of various microplates.

Working from previous knowledge that 275nm and 365nm sources work synergistically to speed RNaseA inactivation, we first used these wavelengths on Greiner Bio-One flat-bottomed 96-well black opaque microplates (Figure 2C). Once efficacy was confirmed on Greiner plates, we began testing microplates of other materials, colors, and well shapes. Each plate first underwent materials testing to determine the intensity of UV light it could withstand. We then determined the minimum intensity of light necessary to successfully inactivate RNaseA in each plate type.

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 you experiment is maintained.

Figure 2A: Tapered Wells

Plate material	Color	Wavelength and power
Polypropylene	White	275nm - 1.0 W/cm^2 375nm - 900 mW/cm^2
Polystyrene	White	275nm - 1.0 W/cm^2 375nm - 900 mW/cm^2
PET	White	275nm - 1.0 W/cm^2 375nm - 900 mW/cm^2
	Black	275nm - 1.0 W/cm^2 375nm - 900 mW/cm^2
Polycarbonate/ polypropylene	Clear	275nm - 1.0 W/cm^2 375nm - 900 mW/cm^2

Figure 2B: Round-Bottomed Wells

Plate material	Color	Wavelength and power
Polypropylene	White	275nm - 1.2 W/cm^2 375nm - 900 mW/cm^2
	Black	275nm - 1.2 W/cm^2 375nm - 900 mW/cm^2
Polystyrene	White	275nm - 1.4 W/cm^2
	Black	275nm - 1.4 W/cm^2
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/cm^2 375nm - 700 mW/cm^2

Inactivation of RNases on Pipettman

Of particular concern for those engaged in RNA work is RNase contamination on pipettes. Unfortunately cleaning chemicals leave residue, often corrode the pipettes that are attempting to clean and are subject to human error. Thus laboratories are in need of a safe, effective method for disinfecting their pipettes.

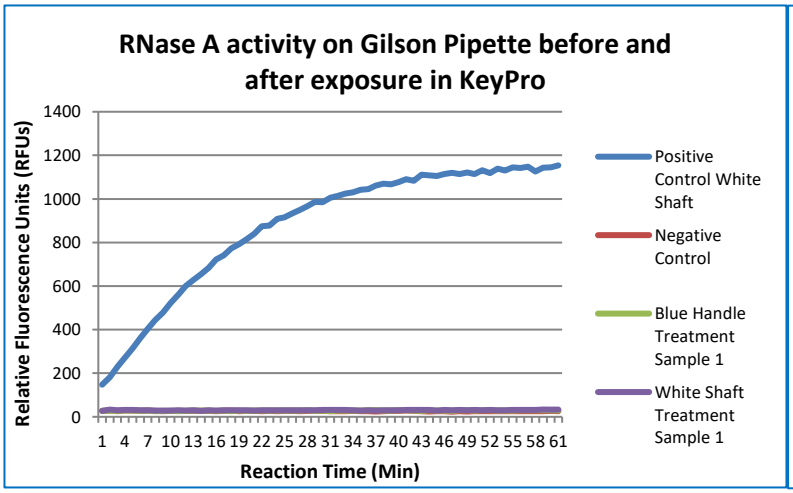
A UV-LED scanning light source (KeyPro™, Phoseon Technology) was used to achieve complete inactivation of RNase A on Gilson pipettes. Exemplified below, the blue handle of a Gilson pipette was intentionally doped with RNase A then treated in KeyPro™ while suspeneded over a specially-designed reflector.

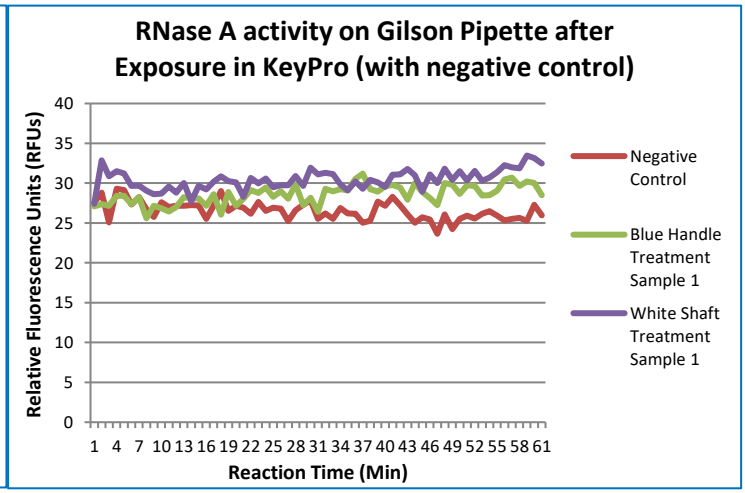
Complete decontamination of the blue handle was achieved in less than ten minutes at 90% 275nm intensity and 70% 365nm intensity.

UV-LEDs are often disregarded when it comes to pipette disinfection due to line-of-sight concerns. Thanks to unprecedented power and effectiveness of the reflector, total inactivation of RNase A on a pipette was made possible with UV-LEDs. Thereby protecting the integriety of your RNA work.

Thanks to never-before-seen power and effective light distribution rapid decontamination of Pipettes is now made possible with UV-LEDs.

A safe, non-corroding form of disinfection.





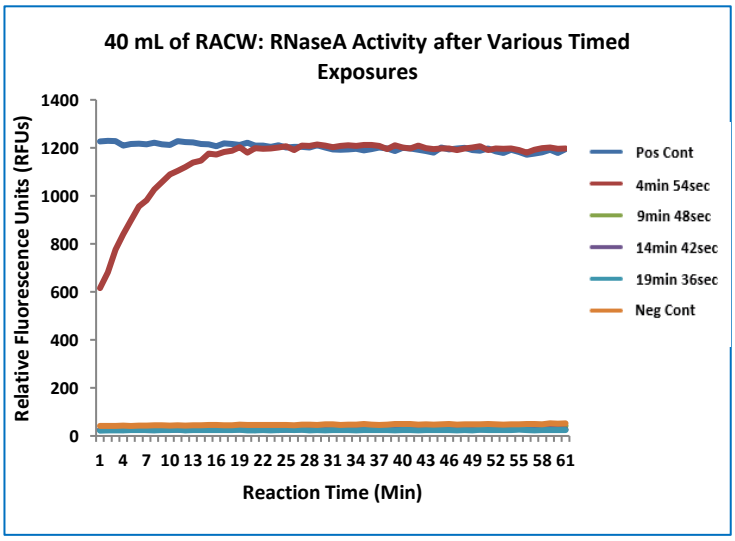
Inactivation in Solution Made Possible with Targeted UV-LED Wavelengths

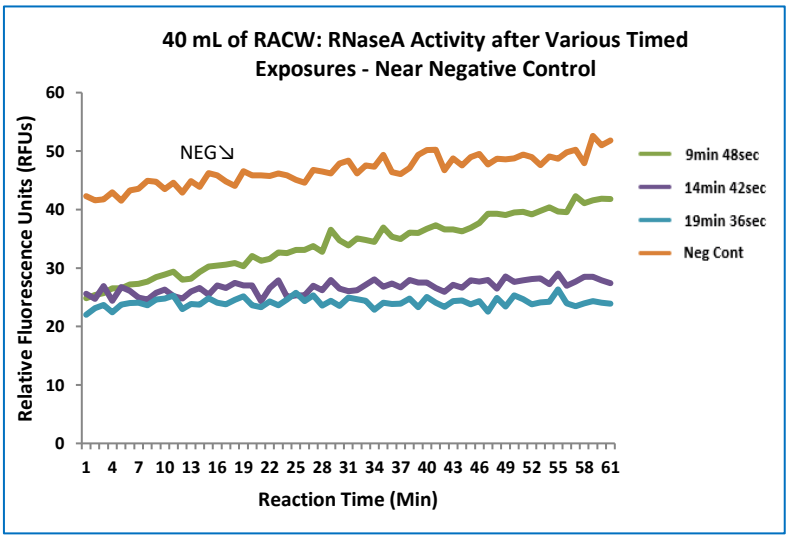
Synergistic, high-powered UV LED wavelengths can be used to generate various amount of RNase-free water at POINT-OF-USE

Combining Phoseon's patented SLM™ technology with well-chosen UV wavelength combinations has not only allowed for effective surface decontamination, but also decontamination in solution. We show here rapid disinfection of RNase-A Containing Water (RACW) using targeted 275 nm and 365 nm light.

Because of power improvements and meticulous wavelength selections we were able to rapidly clean various quantities of RACW (not all sizes represented here). For example, 40mL of RACW was decontaminated in under ten minutes.

This kind of flexibility enables production of clean water at point-of-use. By providing the right amount of clean water for each experiment the laboratory saves both time and money. Limiting the waste of large quantities of water that become contaminated over time and removing the need to purchase small-volumes of clean water at high-cost.





Effectiveness of UV LED for Modulating Enzymatic Activity

Given the targeted nature of LEDs a study was undertaken to assess the differing enzymatic effects of various combinations of LED light on the difficult to inactivate RNase A enzyme.

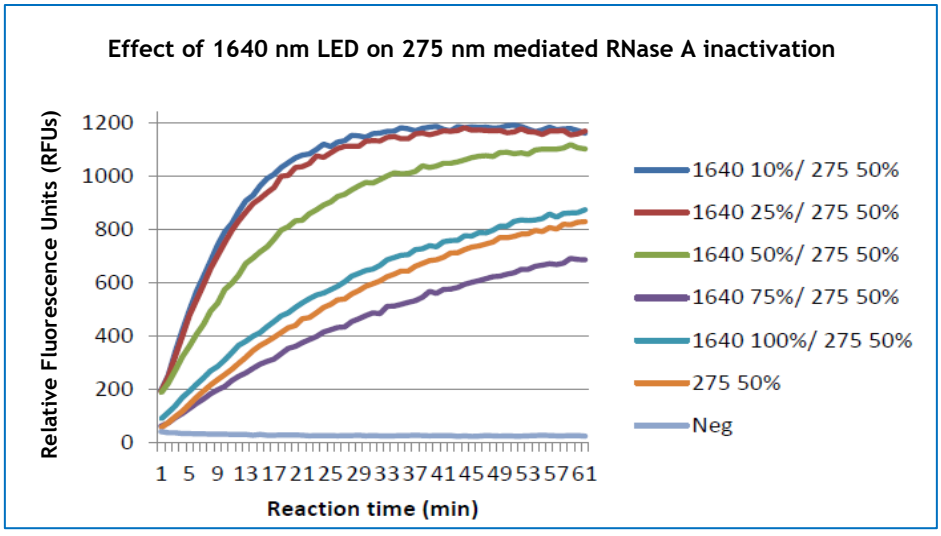
Wavelengths chosen were 275 nm (targets aromatic amino acids proximal to disulfide bonds), 365 nm (approximates the BDE 0f 331 kJ/mol for the CH3-NH2 bond present in the lysine side chain), 340 nm (potential affect on sulfhydyrl groups) and 1640 (potential effect on protein secondary structure, ex:alpha helix melting). Interestingly, the combination of 275 nm with 1640 nm shows a novel and unexpected pattern.

1640 nm light appears to be slowing the reaction down at specific power levels. At 50% 1640 nm power and below the reaction is shifted *above* the positive control (50% 275 nm alone). Representing an increase in enzyme activity. This suggests a level of wavelength-specific effects previously undocumented.

Because of the broad spectrum of mercury lamps used almost exclusively in inactivation and decontmatination studies to date information on the synergistic effects of various wavelengths has been hard to come by. Targeted UV-LEDs are the optimal tool for elucidating wavelength effects, as evidenced by the additional NEG↘ synergies found and novel preliminary findings of enzyme modularity.

Specific wavelengths can be used to modulate reaction rates of RNase, either slowing or speeding it up.

Mercury lamps output a broad spectrum, in which different wavelengths can work AGAINST each other.



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.
- The speed of UV LED inactivation of RNase A allows for rapid production of clean water at point-of-use and disinfection of pipettes
- Wavelengths of 275 nm and 365 nm interact synergistically resulting in faster inactivation, at lower irradiances, than is achievable with either wavelength alone.
- Preliminary results suggest the possibility of modulating enzyme activity with specific, targeted UV wavelengths
- 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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