

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.

UV LEDs Offer New Method for Controlling RNase Contamination

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.

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

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:

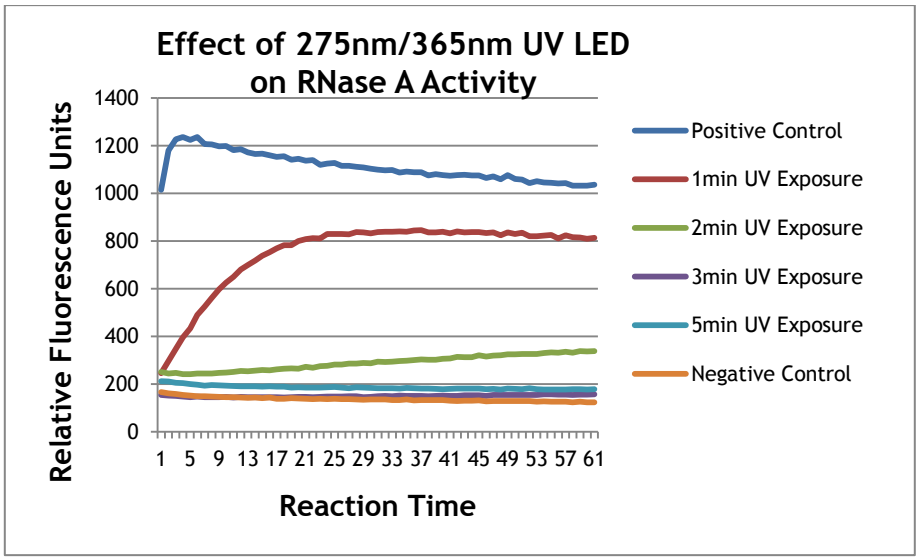
1) *Ultra-low input and Single-cell RNA sequencing*
2) *Ribosome profiling*
3) *RNA Exome Capture sequencing*
4) *Targeted RNA sequencing*
5) *Small RNA sequencing*
6) *Total RNA sequencing*
7) *mRNA sequencing*
8) *CRAC (Crosslinking And cDNA analysis)*
9) *iCLIP (individual-nucleotide resolution Cross-Linking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

Irreversible Inactivation of RNases on Surfaces Now Possible with UV LED

High-intensity UV LED irradiation represents a novel, fast and convenient irreversible inactivation method for RNases on surfaces.

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.



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 an 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.

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.



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

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

For more information about Phoseon Technology suite of products, visit **http://www.phoseon.com/** or call **(503) 439-6446**

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