High-intensity UV LEDs for Point of Use Production of RNase-free Water

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Overview:

- Defined wavelength UV LED exposure can inactivate RNase A in water
- Small volumes can be rapidly inactivated with either static or scanning high-intensity UV LED light sources
- Combined 275 nm and 365 nm UV LED can inactivate RNase A in 100 µl volumes, as dispensed to microtiter wells, in under 5 min
- High-intensity UV LED sources allow point-of-use production for small- and micro-volume amounts of RNase A-free water

Introduction:

RNase-free water is frequently used in cloning and sequencing protocols and a critical need in laboratories engaged in siRNA, RNAi, or microRNA work. It is also easily contaminated and time-consuming to prepare. We have shown that RNase A can be inactivated[1] or modulated[2] using wavelengths targeted to bond dissociation energies of disulfide bonds and lysine side chains. Based on our work using specific synergistic wavelengths of high-intensity UV LEDs[1] to inactivate RNase A on surfaces, we have constructed a scanning UV LED light source with adjustable intensity and speed. This scanning source, in addition to our stationary high-intensity UV LED sources was used for rapid inactivation of RNase A in small volumes of contaminated water — providing point-of-use RNase-free water.

Methods:

Three volumes (100 µl, 40 mls, and 50 mls) of RNase A Containing Water (RACW) at 0.02 u/ml (Worthington) were illuminated with high-intensity UV LEDs.

50 ml Samples: A 50 ml glass beaker of RACW was exposed to an 8W FJ200 275 nm (FireJet™, Phoseon Technology, 278 nm with an irradiance of 2.2 W/cm² at the lamp window). The UV source window was 25 mm from the water surface. Distance to the bottom of the beaker was 75 mm. Samples were taken from the top and bottom levels in the beaker both with and without stirring.

40 ml Samples: A glass petri dish (uncovered) of RACW was placed 1 mm below the scanning UV LED source window (KeyPro™, Phoseon Technology). Maximum irradiance at the scanning window was 2 W/cm² (275 nm) and 1 W/cm² (365 nm), irradiance was adjusted down for testing. Initial scan speed was 0.5 mm/sec.

100 µl Samples: RACW was placed in the wells of a 96-well polypropylene plate. The top of the plate was located 1 mm below the scanning window. Scan speed (KeyPro, Phoseon Technology) was either 0.5 mm/sec or 0.75 mm/sec, irradiance was 275 nm 60% to 80% maximum and/or 365 nm 90% maximum.

Results:

As sample volume increased, the time required for inactivation also increased.

50 ml Samples: RNase A (0.02 u/ml) was inactivated in 30 minutes without stirring (data not shown) and in 20 minutes with stirring.

40 ml Samples: With simultaneous scanning of 275 nm (70% maximum irradiance) + 365 nm (90% maximum irradiance) inactivation was achieved with 2 scans for a total time of 9 min 48 sec. Reducing the irradiance of 275 nm to 60% increased the time required for inactivation to 14 min 42 sec.

100 µl Samples: RNase A is inactivated to levels at or near negative control when exposed to 275nm (80% maximum irradiance) + 365 nm (90% maximum irradiance) scanning at 0.5 mm/sec (4 min 54 sec total scan time).

Conclusions

Small quantities of water can be rapidly rendered free of RNase activity at the point-of-use. In experiments where absence of RNase is critical, this inactivation can be applied after dispensing aqueous components. We anticipate further decreases in the time required to produce small- and micro-volume RNase-free water will parallel an increase in available source irradiance.

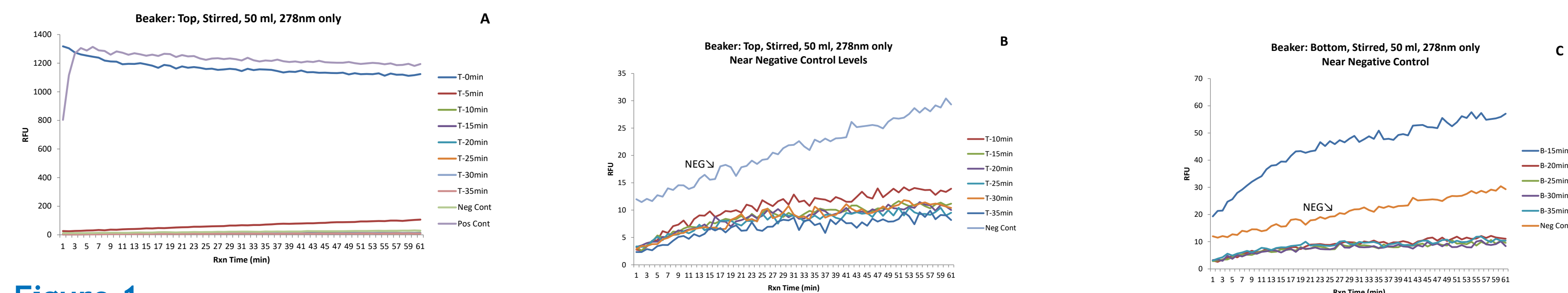

Figure 1

Inactivation of RNase A solution (50ml) in a Griffin beaker.

The sample was exposed to 278 nm UV LED (2.2 W/cm² at the lamp window), from a distance of 25 mm (lamp window to liquid surface). **A)** RACW was exposed to 278 nm UV LED for 0 min, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min and 35 min. Samples (20 µl) were taken from the top and bottom of the beaker, then the solution was stirred by hand for 30 sec before the exposure to UV LED was continued. RNase A activity was assayed with RNase Alert (IDT) and the fluorescence read on a Gemini XPS fluorimeter (Mol. Devices). **B)** An expanded view of samples taken from the top of the solution. **C)** An expanded view of samples taken from the bottom of the beaker.

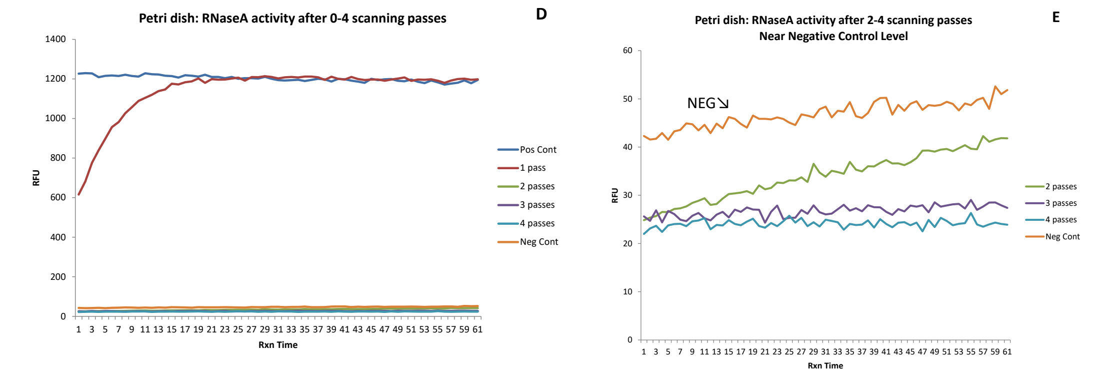

Figure 2

Inactivation of RNase A solution (40ml) in an open top glass petri dish.

The sample was scanned with combined 278 nm + 365 nm high-intensity UV LED at 0.5 mm/sec. **D)** RNase A activity for 0 through 4 scanning passes. **E)** An enlargement of Graph D showing RNase A activity for 2 though 4 scanning passes as compared to the negative control.

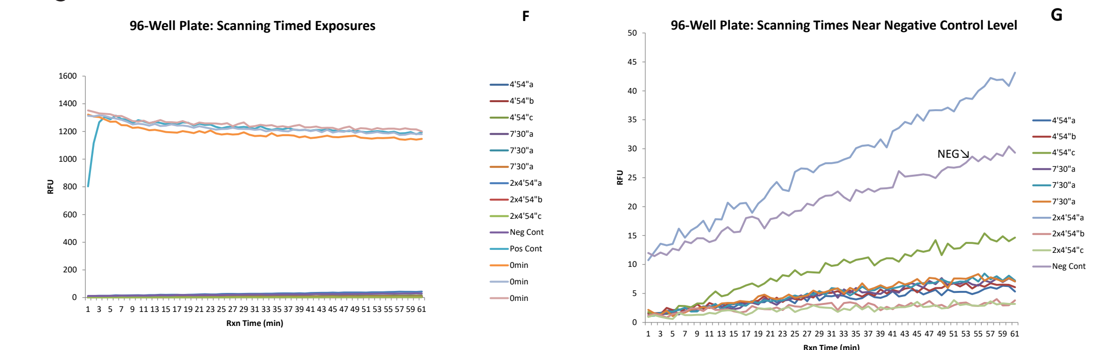

Figure 3

Inactivation of RNase A solution (100 µl/well) in a 96-well black opaque plate.

The sample was scanned with 278 nm high-intensity UV LED for no exposure (positive activity control), 0.5 mm/sec, 0.75 mm/sec, and 2 x 0.5mm/sec. **F)** RNase A activity for triplicate scans. **G)** An enlargement of Graph F showing RNase A activity for 0.5 mm/sec, 0.75 mm/sec, and 2 x 0.5mm/sec scanning passes as compared to the negative control.

Literature Cited

Thompson T, Eliason G, Pasquantonio J. Synergy between 275 nm and 365 nm UV LEDs for inactivation of RNase A. Poster presented at: SLAS. 2018 Feb 3-7. San Diego, CA.

Thompson T, Taggard K, Pasquantonio J. Enzyme activity modulation by LED and UV LED. Poster presented at ASM. 2018 June 7-11. Atlanta, GA.

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