Quantification of Ambient RNase A Contamination and Inactivation in Various Common Microplates

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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.

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.

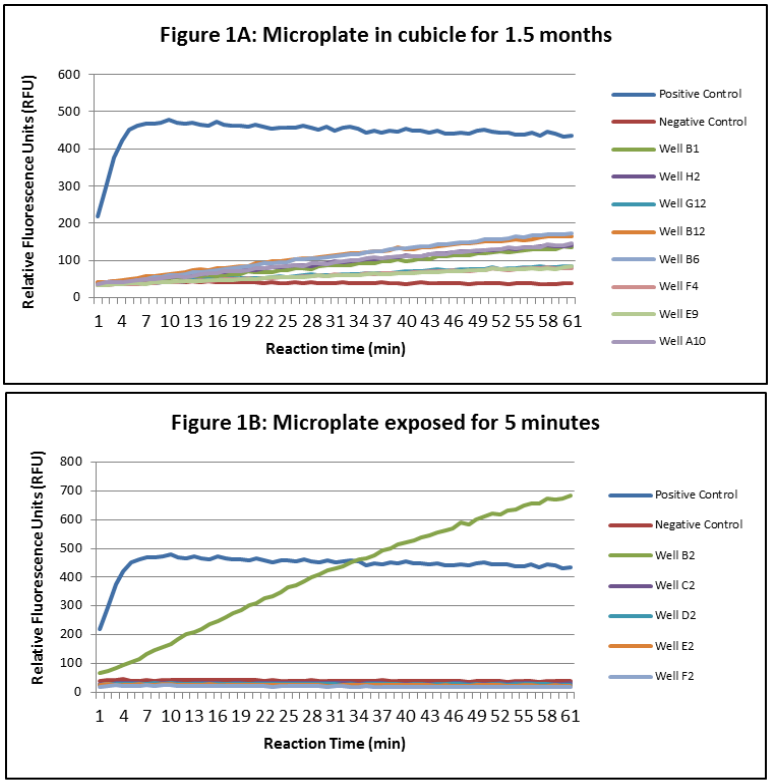

Figure 1: Long-term vs short-term ambient contamination exposure
A flat-bottomed, black opaque microplate was exposed to an office cubicle environment for 1.5 months (a) and a second microplate was exposed to an office lunch-room environment for 5 minutes (b). Sample wells were suspended and fluoroimetrically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

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.

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.

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

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

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).

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.

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Work Cited:

1.) Thompson T, Taggard K, Pasquantonio J. (2018, June) *Enzyme Activity Modulation by LED and UV LED* Poster session presented at the Annual American Society for Microbiology Microbe 2018 conference, Atlanta, GA