Inactivating RNase A in Water for Lab Use

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Nuclease-free water is vitally important for accurate results in a number of laboratory experiments. Whether the research is cellular or sequencing, the application is drug discovery or forensics, nuclease-free water is used for extracting biomolecules from a sample without the risk of enzymatic damage to the DNA and RNA components. Nuclease-free water is also expensive: sometimes over $5 USD per mL, depending on the volume.

The most difficult enzyme to eliminate in the laboratory environment is RNase. It is ubiquitous in the environment, resists thermal and chemical inactivation, and can significantly degrade precious RNA samples in minutes.

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 NGS or looking at individual RNAs (iCLIP), degradation by RNases is a recurring laboratory handling issue requiring stringent cleaning methods.

Most experiments only need small volumes of nuclease-free water at a time. Typical experiments require 100 mL or less of nuclease-free water for sample extraction and aliquoting. So, we examined whether RNase A can be inactivated in water, on demand, using high-intensity ultraviolet light generated by UV LEDs.

UV light exposure requires a direct path between the light source and the full volume of liquid. We decided against using vessels that are good transmitters of deep ultraviolet light, as such materials are rare in most labs. In order to represent common equipment, we ran experiments using standard beakers, petri dishes, and microplates as the vessels for inactivating RNase-doped water under a high-intensity UV LED array.

50 mL Beaker:

A 50 mL beaker was filled with 50 mL of nuclease-free water with 0.02 units/mL RNase A. The water was treated with a combination of 275 nm and 365 nm high-intensity light at a distance of 25 mm from the emitting window. At 5 minutes of exposure, approximately 40% of RNase A activity remained (RNase Alert™ assay quantified on a Molecular Devices Gemini XPS fluorimeter). At 10, 15, and 20 minutes of exposure, RNase A activity was at or below the negative control.

Petri Dish:

Petri dishes offer the advantage of a large surface area exposed to the ultraviolet source relative to the volume of water being treated. For experiments with Petri dishes, the UV source was the KeyPro

KP100 decontamination instrument. The KeyPro KP100 uses a high-intensity dual-wavelength light source (275 nm/365 nm) that scans across the emitting window. For each experimental run, an open top glass Petri dish with 40 mL of RNase-contaminated water (as described above) was placed within 2 mm of the emitting window of the instrument. Sequential 5-minute passes of the UV LED array were run for different Petri dishes. Intensity of the two wavelengths was set at 70% for 275 nm and 90% for 365 nm. A single 5 minute pass did not completely inactivate RNase A. Passes 2 through 4 showed complete inactivation of RNase (RNase activity equal or less than negative control). A second experiment was performed with 275 nm alone, set at 100% intensity. While multiple passes of exposure decreased RNase activity, even 4 passes of 275 nm alone did not reduce RNase activity to the level of the negative control.

Microplates:

Microplates are workhorses for biological research and development. For assays that require nuclease-free water, a workflow-friendly method for sample prep is to pre-load microplate wells with the desired volume of water for sample extraction. Then the extracted sample volume can be returned to the initial well. Success, however, depends on the quality of the water in the well. In this study, standard 96-well microplate wells were inoculated with 100 microLiters of water containing 0.02 units/mL of RNase A. The KeyPro KP100 was used to treat the microplates with 70% intensity of 275 nm and 90% intensity of 365 nm and a scan speed of 0.5 mm/sec. RNase A activity in each treated well was at or below the negative control in a single scan.

These experiments show that small quantities of water can be rapidly and reliably decontaminated of active RNase on demand. This application may be very useful for labs that frequently use small quantities of nuclease-free water.

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