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BioTek

Application Bulletin

Quantification of RNA using Microplate-based UV and Fluorescence Methods

Introduction

There has been a dramatic shift in our understanding of the role performed by various forms of ribonucleic acids (RNAs) recently. New activities are being discovered performed by various RNAs across a variety of biological pathways in cell based organisms, including regulatory RNAs such as miRNAs, siRNAs piRNA and CRISPR RNAs as well as the more traditional RNAs associated with translation (mRNA, tRNA and rRNA) and RNA processing (snRNA and snoRNA). There continues to be increased interest in research efforts targeted to better understand the multifaceted roles RNAs play. One central requirement for many experimental systems is the quantification of RNA prior to use. Several methods to quantify RNA are available including the use of the intrinsic absorption of light by RNA in the ultra-violet wavelength range as well as enhancement reagents allowing greater sensitivity and specificity when contaminating species may be present.

Methods

UV Absorption

All ribosomal RNA standards were created by preparing a 1:2 serial dilution series of a concentrated stock in TE buffer (tris-EDTA, pH=7.0). Absorbance measurements were performed at 260 nm in triplicate in a 100 µL volume in a UV-transparent microplate.

Fluorescence Assay

Quant-iT™ RiboGreen Reagent, cat # R11490, was purchased from Thermo-Fisher. Solid 96-well black microplates, (cat # 3915), were from Corning. Ribosomal RNA was diluted to 2 µg/mL with TE (10 mM Tris, 1 mM EDTA, pH 7.5) as the diluent. The final concentration was confirmed using 260 nm absorbance. A series of dilutions ranging from ~0.0 to 3,200 ng/mL or 0.0 to 100 ng/mL of purified RNA were made using TE and 100 µL aliquots pipetted into microplate wells. Equal amounts (100 µL) of working RiboGreen quantitation reagent were mixed and incubated for 10 minutes at room temperature, protected from light. Working RiboGreen reagent was prepared by diluting the concentrated DMSO-RiboGreen stock solution 1:200 with TE according to the manufacturers' recommendations. Fluorescence was determined using a Synergy™ LX Multi-Mode Reader with a GFP filter cube (EX 485/20 nm, EM 528/25 nm and 510 nm cutoff dichroic mirror). Concentrations are reported as those in the final assay volume.

Results and Discussion

UV absorbance remains the most common method for estimation of nucleic acid concentration primarily due to ease of use. Of primary concern is over-estimation due to contaminating species with identical absorption peaks. Additionally, absorption methods can suffer elevated limits of detection that is most notable when working with samples in the sub-microgram per milliliter range. However, UV absorbance provides a quick estimate of purified RNA across a relatively large range of concentrations commonly resulting from many laboratory preparations (Figure 1).

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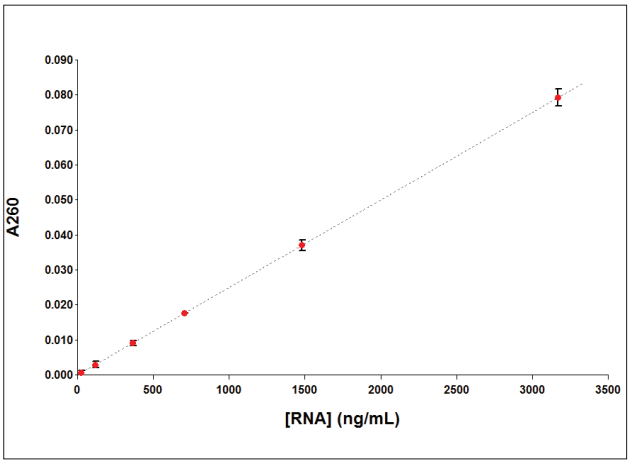

Figure 1. **RNA absorbance measurements.** A dilution series of ribosomal RNA was prepared ranging from 0 – 3,200 ng/mL in TE buffer. Standards were analyzed in triplicate at a volume of 100 µL in a UV transparent, 96-well microplate format.

The use of fluorescent intercolating dyes can effectively be used to accurately quantify lower concentration samples as shown below. These dyes were developed to be able to discriminate between different biomolecules and, to some extent, different nucleic acid species. The RNA assay can be run in either a high or low range format by adjusting the reagent concentration to either a 200 or 2,000-fold dilution, respectively (Figures 2 and 3). The calculated limit of detection for the high- and low-range assays are nearly identical at 0.5 and 1.7 ng/mL, respectively.

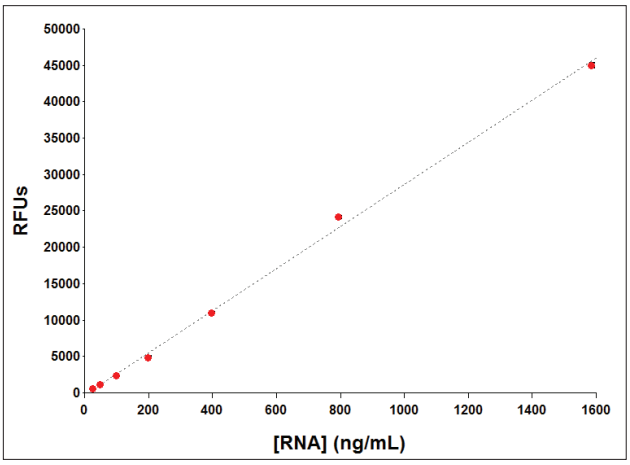

Figure 2. **High range fluorescence measurements.** A dilution series of ribosomal RNA was prepared ranging from 0 – 3,200 ng/mL in TE buffer. Standards were analyzed in triplicate at a volume of 100 µL in a standard 96-well microplate format.

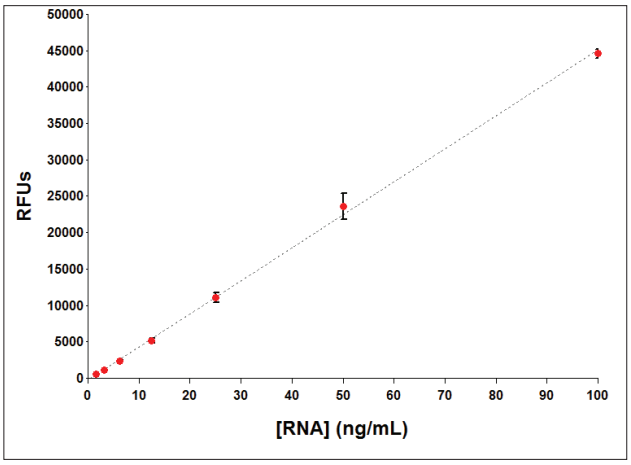

Figure 3. **Low range measurements.** A dilution series of ribosomal RNA was prepared ranging from 0 – 100 ng/mL in TE buffer. Standards were analyzed in triplicate at a volume of 100 µL in a standard 96-well microplate format.

Conclusions

The Synergy™ LX Multi-Mode Reader provides the most common detection technologies used in biological research including absorbance, fluorescence and luminescence detection. Here we demonstrated its utility to quantify proteins using UV detection and various colorimetric assays to extend the dynamic range of measurable concentrations and possessing tolerance to many interfering compounds.