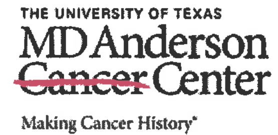
Date: July 18, 2018



Department of Genomic Medicine

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Division of Cancer Medicine

The University of Texas MD Anderson Cancer Center

Director:

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SOP RiboGreen – Detection of RNA on the BioTek Synergy™ Microplate Reader

To Prepare the Reagents:

- Remove reagents from the fridge and allow them to warm to room temperature.
    - The dye reagent is light-sensitive, so keep it in the reagent bag before and after use.
- Dilute 20X TE Buffer to 1X (50µl + 950µl H_2O) – make sure to check for aliquots in the fridge (they're good for 1 week)
    - 3.5mL of 1X TE will be used for each standard curve and an additional 1mL will be need for each sample.
- To prepare the RiboGreen reagent, add 100µl of concentrated DMSO and 19.9mL 1X TE (for 20 samples).

To Prepare the Standards (one set per run):

- Using the RNA stock provided in the RiboGreen kit (100µg/ml), prepare a 2µg/mL standard by adding 40µl of the stock RNA (100µg/ml) and 1.96mL 1X TE.
- Five standards will need to be prepared for RNA quantitation:

Standard No. (ng/ml)	Amount of 2µg/mL RNA Stock (ul)	Volume of 1X TE (ul)	Volume of RiboGreen Reagent (ul)
STD1 (1000)	1000	-	1000
STD2 (500)	500	500	1000
STD3 (100)	100	900	1000
STD4 (20)	20	980	1000
STD5 (-)	0	1000	1000

To Prepare the Samples:

- Prepare a 1:1000 dilution of each sample by adding 1µl of stock RNA sample and 999µl of 1X TE.
    - Make sure to include a blank that contains 1mL 1X TE for blank subtraction.
- Add 1mL RiboGreen Reagent to each dilution.
- Cover the standards and samples with a foil lid and turn off the bench overhead light – let the samples incubate for 2-5 minutes at room temperature.

To Prepare the Microplate for Analysis:

- Aliquot 200ul of each standard and sample in the microplate wells – place according to the sample layout specified on the Synergy instrument.

Operation of the BioTek Synergy™ Microplate Reader:

- Open the Gen5 software, click Instrument Control, and select Control Lamp to warm the fluorescent bulb.
    - Make sure to turn the bulb off when not in use – it will automatically shut off after 4 hours

Running the DNA Samples

- Select File -> New Task -> Experiments -> Create using an existing protocol -> RNA Quantitation using RiboGreen.
- Select Plate Layout, right-click and select Empty Layout.
- Add the standards, blanks, and samples according to the plate layout.
- To run the plate, select the green play button at the top. Follow the prompts as directed.

Data Analysis & Export

- Click on the Graphs tab and verify that the R^2 value is > 0.95.
- Click on the Statistics tab and select Concentration from the Data drop-down menu.
- Right-click, select QuickExport, and choose the location and save the file.

*Make sure to keep a key of the samples included in the plate layout as the sample IDs cannot be changed in the software.

Genomics Laboratory

Curtis Gumbs, Scientific Manager, Genomic Medicine

7/13/18

[Signatures] and [dates]