Quant-iT™ RiboGreen™ RNA Quantification V.3

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Version 3
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1 Works for me
dx.doi.org/10.17504/protocols.io.bfbxjipn

SoWa RI Anaerobic and Molecular Microbiology (public)
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ABSTRACT

The following protocol is intended for the quantification of RNA using Quant-iT™ RiboGreen™ RNA Assay Kit (ThermoFisher). This protocol is a simplified and condensed version of the full protocol from the manufacturer. The procedure described here is for 96 reactions. If samples are run in duplicates, then this should allow quantifying 40 samples.

EXTERNAL LINK

https://sfamjournals.onlinelibrary.wiley.com/doi/abs/10.1111/1462-2920.12140

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Angel, R., and Conrad, R. (2013). Elucidating the microbial resuscitation cascade in biological soil crusts following a simulated rain event. Environ Microbiol 15, 2799–2815. doi:10.1111/1462-2920.12140.

ATTACHMENTS

mp11490.pdf

MATERIALS

NAME	CATALOG #	VENDOR
Quant-iT™ RiboGreen™ RNA Assay Kit	R11490	Invitrogen - Thermo Fisher

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Quant-iT™ RiboGreen® RNA Assay Kit	R11490	Thermo Scientific

EQUIPMENT

NAME	CATALOG #	VENDOR
Infinite M Nano	TEC006436I	
Multipette® E3	4987000010	
96-Well microtiter plate	165305	

SAFETY WARNINGS

Quant-iT™ RiboGreen® RNA reagent is classified as Not Hazardous. Nevertheless, the user should always consult the MSDS accompanying any of the reagents and apparatus described in this protocol.

BEFORE STARTING

1. This protocol is optimised for measuring an entire 96-well plate. It assumes that 10 wells will be used for measuring the standards and the blank samples (in duplicates) and 86 wells will be used for measuring unknown RNA samples (typically in duplicates).
2. The protocol can be easily adjusted for a lower number of samples by reducing the volume of the working solutions of the reagents. Note though that enough TE should be retained for diluting the standard stock solution (490 or 680 µl), for potentially diluting the unknown samples, if their concentration is too high, and for

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accounting for pipetting errors. To fill the plate, 19.2 ml of TE is needed. So if only 40 wells are to be used for measuring unknown samples prepare about $50/96 \times 22 \approx 11.5$ ml of TE buffer.

3. The dynamic range of the assay is between 1 ng ml^{-1} to 50 ng ml^{-1} in the "low-range" version of the assay and 20 ng ml^{-1} to 1 µg ml^{-1} in the "high-range" version of the assay. This translates into RNA sample concentrations of 0.2-10 ng $µl^{-1}$ and 4-200 ng $µl^{-1}$ in the low-range and high-range assays, respectively. Samples with higher RNA concentration need to be diluted (e.g. in RNase-free water or TE buffer).
4. Note that some compounds that often contaminate RNA are known to interfere with the measurement and produce a lower observed measurement. Please refer to the full protocol for a list of these compounds and their effect on the measurement.
5. Quant-iT™ RiboGreen® reagent also binds to DNA. Samples containing DNA should be pre-treated with an RNase-free DNase before using this protocol to ensure that the fluorescent signal is solely due to binding with RNA.

Prepare the reaction

1 Take out all reagents from the fridge and bring them to room temperature. 20m
Take out the RNA samples from the freezer. RNA samples should be slowly thawed on ice.

Quant-iT™ RiboGreen® RNA reagent is dissolved in dimethylsulfoxide (DMSO), which freezes below 19 °C. The reagent must be completely thawed before using it by bringing it to room temperature. After the reagent thawed, it is advisable to briefly vortex the tube to make sure it is adequately mixed and to spin it down in a centrifuge or a mini centrifuge.

Quant-iT™ RiboGreen® RNA reagent is light sensitive and should be protected from light at all times.

Quant-iT™ RiboGreen® RNA Assay Kit
by Thermo Scientific
Catalog #: R11490

2 Prepare 22 ml 1X TE buffer by pipetting 1.1 ml of 20X TE buffer into 20.9 ml of nuclease-free water into a sterile and nuclease-free 50 ml tube. 2m
Mix by inverting the tube several times.

1.1 ml 20X TE buffer

20.9 ml nuclease-free water

3 **For high-range quantification:** 2m

Dilute the *E.coli* rRNA-standard stock solution (100 µg ml^{-1}) to a final concentration of 2 ng $µl^{-1}$ by mixing 10 µl of rRNA-standard stock solution with 490 µl 1X TE buffer.

10 µl rRNA-standard stock solution

490 µl 1X TE buffer

For low-range quantification:

Prepare a 20-fold dilution of the 2 ng $µl^{-1}$ rRNA-standard work solution by mixing 10 µl of the 2 ng $µl^{-1}$ rRNA-standard work solution with 190 µl 1X TE buffer to yield 0.1 ng $µl^{-1}$ rRNA-standard work solution.

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10 µl diluted rRNA-standard solution

190 µl 1X TE buffer

4 If needed, prepare a dilution of each sample in 1X TE so that the reading will be within the dynamic range.

It is advisable to run samples in duplicates for a more accurate quantification

2m

5 Prepare the RiboGreen® work solution:
For the **high-range assay** pipette 50 µl Quant-iT™ RiboGreen® RNA Reagent and 9950 µl of 1X TE into a sterile and nucleic-acids free 50 ml tube.
For the **low-range assay** pipette 5 µl Quant-iT™ RiboGreen® RNA Reagent and 9995 µl of 1X TE into a sterile and nucleic-acids free 50 ml tube.

Mix by inverting and keep the solution away from light.

10m

6 Prepare one of the following standard mixtures in the first two columns of a black, sterile, 96-well plate:

Assay version	Diluted RNA std. (µl)	1X TE (µl)	Final RNA amount (ng)
High-range (4-200 ng µl-1)	100	0	200
Use 2 ng µl-1 standard	50	50	100
	10	90	20
	2	98	4
	0	100	0
Low-range (200 pg µl - 10 ng µl-1)	100	0	10
Use 0.1 ng µl-1 standard	50	50	5
	10	90	1
	2	98	0.2
	0	100	0

96-Well microtiter plate
Polystyrene cell-culture plate
Nunc 165305
Black, flat-bottom, sterile

2m

7 Pipette 99 µl of 1X TE buffer in the remaining wells.

99 µl 1X TE buffer

Tip: use a mechanical or electronic dispenser during this step and step no. 9 to speed up the work.

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Multipette® E3
Electronic dispenser
eppendorf 4987000010

Step 7 includes a Step case.
For low-concentration samples

step case

For low-concentration samples

To measure samples with a low concentration, it is possible to reduce the volume of the work solution at this step and pipette more sample in the next step, for a total volume of 100 µl.

8 Pipette 1 µl of the unknown RNA samples in the remaining wells. 5m
1 µl Unknown RNA sample

Use either a diluted sample in case the concentration is expected to be higher than the dynamic range limit or larger volume in case the concentration is expected to be below the detection limit.

9 Pipette 100 µl of the RiboGreen® work solution into each well, including the standard and unknown sample wells 2m
100 µl RicoGreen work solution

10 Protect the 96-well plate from light and incubate for 2-5 min at room temperature. 5m
00:05:00

Measure the samples 5m

11 Place the plate in a plate reader and measure the fluorescence according to the following parameters: 5m

Excitation	~480 nm
Emission	~520 nm
Integration time	40 s
Lag time	0 s
Gain	Optimal
Number of flashes	10
Calculated well	highest standard
Shaking	5 s

Infinite M Nano
Absorbance plate reader
Tecan TEC006436I

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It is also possible to set the gain to a fixed value (e.g. 100). If the fluorescence values of the standard drop over time this could indicate damage to the reagents or the RNA standard.

12 Plot the measured fluorescent values of the standard samples against their known concentrations and fit a linear curve using linear regression. Make sure that the coefficient of determination (R^2) is close to 1 (typically >0.98). Calculate the RNA concentrations in the unknown samples using the slope and intercept parameters of the linear equation. Output values you obtained are in ng μl^{-1}, assuming 1 µl of each sample was used. 10m

Do not forget to account for any dilutions when calculating the concentration of the RNA in the unknown samples

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