

# RiboGreen Quantification using a qPCR machine

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## Method Article

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# Abstract

This protocol describes how to use a qPCR machine to perform RiboGreen quantification of the concentration of extracellular RNA in a sample.

## Introduction

Extracellular RNAs (exRNAs) have been identified in every biofluid that has been tested. They have been found in extracellular vesicles, ribonucleoprotein complexes and lipoprotein complexes. exRNAs are interesting because they may serve as signalling molecules between cells, they have the potential to serve as biomarkers for prediction and diagnosis of disease, and exRNAs or the extracellular particles that carry them might be used for therapeutic purposes. The Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium (ERCC) is a group of laboratories funded by the U.S. National Institutes of Health to develop robust and standardized methods for collecting and processing of biofluids, separating different types of exRNA-containing particles and isolating and analyzing exRNAs. In our first joint endeavour, we held a series of conference calls and in-person meetings to survey the methods used among our members, placed them in the context of the current literature and used our findings to identify areas in which the identification of robust methodologies would promote rapid advancements in the exRNA field. A full list of the protocols developed during this effort is available at the exRNA Portal, the ERCC's website (<http://exrna.org/resources/protocols/>). This protocol for performing RiboGreen quantification is one of the final steps in the process, allowing quantification of the amount of extracellular RNA found in a sample or biofluid.

## Reagents

Quant-It RiboGreen Assay Kit\* (Life Technologies, catalog # R11490)

## Equipment

qPCR machine  
96-well plates compatible with qPCR machine

## Procedure

1. Dilute 20X TE (Component B) 1:20 in RNase-free water to make 1X TE Buffer.
2. Make standards in microfuge tubes using the 100 µg/mL rRNA standard included in the Quant-It RiboGreen Assay Kit. These can be stored for future use. [See figure in Figures section.](#)
3. Based on the expected concentrations of the samples, make the appropriate dilution of the RiboGreen reagent (Component A) in 1X TE (1:400 for samples between 2-100 ng/µL, 1:4,000 for samples between 0.05-1 ng/µL). You will need 20 µL of the diluted reagent for each sample and each standard.
4. Based on the expected concentrations of the samples, set up the assay plate with 1 µL each of the appropriate standards.
5. In each well, mix 1 µL of

sample/standard with 19  $\mu\text{L}$  of the appropriate dilution of the RiboGreen reagent. Pipet up and down to mix. 6. Read in a qPCR machine (use FAM/SYBR channel). Export raw fluorescence values. Draw the standard curve, and use the linear trend line to generate the formula to calculate the concentration for each sample.

## Troubleshooting

\*\* Please note that you **must** use the Quant-It RiboGreen Assay Kit, as listed above, **NOT** the Quant-It RNA Assay, Broad Range, as the Broad Range kit uses a different dye in the far-red range which cannot be detected by a qPCR machine.

## Acknowledgements

This protocol was modified from the manufacturer's instructions for the Quant-It RiboGreen assay kit.

## Figures

Concentration of standards	Stock solution used	Volume stock solution (ul)	volume 1X TE (ul)	Final concentration in 20 ul assay	Dilution of the RiboGreen working solution to use
100 ng/ul	This is the RNA standard (100 $\mu\text{g}/\text{mL}$ = 100 ng/ul)			5 ng/ul = 5 $\mu\text{g}/\text{mL}$	1:400
50 ng/ul	100 ng/ul	100	100	2.5 ng/ul = 2.5 $\mu\text{g}/\text{mL}$	1:400
10 ng/ul	100 ng/ul	20	180	0.5 ng/ul = 500 ng/mL	1:400
2 ng/ul	10 ng/ul	40	160	0.1 ng/ul = 100 ng/mL	1:400
1 ng/ul	10 ng/ul	20	180	0.05 ng/ul = 50 ng/mL	1:4,000
0.5 ng/ul	1 ng/ul	100	100	0.025 ng/ul = 25 ng/mL	1:4,000
0.1 ng/ul	1 ng/ul	20	180	0.005 ng/ul = 5 ng/mL	1:4,000
0.05 ng/ul	1 ng/ul	10	190	0.001 ng/ul = 1 ng/mL	1:4,000

Figure 1

Table 1 rRNA Standard Dilution Series