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Low Abundance RNA Quantification Kit — Product Insert

Product # 58900

The amount of RNA that can be extracted from different biological or clinical samples varies greatly. For example, while a few micrograms of RNA could be easily purified from tissues and cells in excess amount (such as from a few milligrams of tissue), many liquid biopsy samples may yield very low amounts of RNA. In fact, samples such as urine or plasma may yield 1 - 100 ng or less RNA per 100 µL of sample. The most commonly used technique for measuring RNA concentration is the determination of absorbance at 260 nm (A260). However, even with the new generation of spectrophotometers, the detection limit of this method is still above 2 - 10 ng per µL. Additional technologies, such as the use of fluorescent nucleic acid stains, has enabled the quantification of RNA at the lower ng or sub-ng per µL range. However, this may not completely overcome the difficulties in quantifying RNA from liquid biopsies where the expected RNA yield could be in the lower pg or sub-pg per µL range.

Norgen's Low Abundance RNA Quantification Kit offers a PCR-based detection procedure to quantify RNA of a wide spectrum of concentrations, including the lower ng per µL, pg per µL and sub-pg per µL range. The kit has two main enzymatic components – reverse transcription using Norgen's microScript Reverse Transcription system and Real-Time PCR Master Mix used in conjunction with a specially formulated primer mixture, to amplify human RNA from different types of inputs (such as various liquid biopsies). The unknown RNA is accurately quantified using a standard curve constructed from the provided RNA Standards on a Real-Time PCR System.

Specifications

Component	Product # 58900
microScript microRNA Enzyme Mix	50 µL
2x microScript Reverse Transcription Master Mix	0.5 mL
2X Real-Time PCR Master Mix	1 mL
RNA Quantification Primer Set Mix	200 µL
Quantified RNA Standards[1]	6 Standards, each 100 µL
Nuclease-Free Water	2 x 1.25 mL
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[1]Quantified RNA Standards are provided as 100 ng/µL, 10 ng/µL, 1 ng/µL, 100 pg/µL, 10 pg/µL and 1 pg/µL

Storage Conditions

Upon receipt, store Norgen's Low Abundance RNA Quantification Kit at -20°C or lower. Avoid multiple freeze-thaw cycles. If needed, prepare smaller working aliquots and store at -20°C or lower.

Quality Control

In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Low Abundance RNA Quantification Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's Low Abundance RNA Quantification Kit is designed for research purposes only. It is not intended for human or diagnostic use.

Customer-Supplied Reagents and Equipment

- Appropriate Real-Time PCR Instrument
- Nuclease-Free PCR Tubes compatible with PCR Instrument
- RNA Purification Kit
    - The kit is compatible with all RNA purification kits that yield high quality, inhibitor-free total RNA (including microRNA)
    - **Recommended Purification Kit:** Norgen Biotek's purification kits for RNA isolation, including:
        - Plasma/Serum RNA Purification Mini Kit - Cat# 55000
        - Urine Cell-Free Circulating RNA Purification Mini Kit - Cat# 56900
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- PCR tubes
- Ice

Warnings and Precautions

- Follow universal precautions. All patient specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.
- Use sterile pipette tips with filters. Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- As contamination of patient specimens or reagents can produce erroneous results, it is essential to use aseptic techniques. Pipette and handle reagents carefully to avoid mixing of the samples.
- Do not use supplies and equipment across the dedicated areas of i) specimen extraction, ii) reaction set-up and iii) amplification/detection. No cross-movement should be allowed between the different areas. Personal protective equipment, such as laboratory coats and disposable gloves, should be area specific.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- The presence of PCR inhibitors may cause invalid results.
- Good laboratory practice is essential for the proper performance of this kit. Ensure that the purity of the kit and reactions is maintained at all times, and closely monitor all reagents for contamination. Do not use any reagents that appear to be contaminated.

Instructions for Use

A. Sample Preparation

Purified RNA is the starting material for Norgen's Low Abundance RNA Quantification Kit. The quality of the RNA template will have a major impact on the performance of the quantification test. We recommend the use of Norgen's purification kits for RNA isolation, including **Norgen's Plasma/Serum RNA Purification Mini Kit (Cat# 55000) and Urine Cell-Free Circulating RNA Purification Mini Kit (Cat# 56900)**. It is highly recommended to perform a DNase treatment on the sample to ensure accurate RNA quantification.

If using a different spin column based sample preparation procedure that includes ethanol-based wash buffers, a column drying step consisting of centrifugation for 10 minutes at 14,000 x g (~14,000 RPM), using a new collection tube, is highly recommended prior to the elution of the RNA. This will help to prevent the carry-over of any ethanol into the purified RNA, as ethanol is known to be a strong inhibitor of PCR. **Ensure that any traces of ethanol from the sample preparation steps are eliminated prior to the elution of the RNA.**

B. Procedure for First-Strand microRNA cDNA Synthesis

- The amount RT-PCR Reagents provided is enough for up to 96 reactions (including sample RT-PCR and standard curve RT-PCR).
- To avoid any contamination while preparing the assay, follow the order outlined in Tables 1 and 2 below to prepare the Sample Assay and Standard Curve
    1. Prepare the Sample Reverse Transcription (Table 1)
    2. Prepare Standard Dilution Series Reverse Transcription (Table 2)
- To further avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) Master Mix; 3) Enzyme; and 4) the Sample RNA or Standards).

1. The procedure could be used for as little as 500 fg total RNA or enriched microRNA. It is highly recommended that RNA is isolated by methods that recover microRNAs. Norgen Biotek provides an extensive line of RNA extraction products that recover all sizes of RNA including microRNAs, without the use of inhibitory chemicals such as phenols. A list of recommended kits is provided at the end of this insert or can be found at *www.norgenbiotek.com*.

2. Thaw the RNA template, RNA Standards, Nuclease-Free Water and 2x microScript Reverse Transcription Reaction Mix on ice. The microScript microRNA Enzyme Mix should be kept at -20°C at all times until just before adding to the reaction mix, and should be returned to -20°C immediately.

3. Set up the First-Strand cDNA Synthesis reaction for the RNA samples to be quantified in a tube compatible with the thermocycler to be used, as described in **Table 1**. Mix the components by gentle vortexing, or by pipetting up and down a few times.

Table 1. First-Strand cDNA Synthesis Reaction Set-up for RNA samples

Component	Volume per Reaction
Nuclease-Free Water	3.5 µL
2x microScript Reverse Transcription Master Mix	5 µL
microscript microRNA Enzyme Mix	0.5 µL
RNA to be quantified	1 µL
Total Volume	**10 µL**

* Up to 4.5 µL of RNA sample could be used per assay. If a volume of >1 µL RNA is to be used, maintain the 10 µL reaction volume by reducing the amount of nuclease-free water to be used accordingly. Note the volume of RNA used for subsequent standard curve quantification

4. Set up the First-Strand cDNA Synthesis reaction for each of the six RNA standards (provided) in a tube compatible with the thermocycler to be used, as described in **Table 2**. Mix the components by gentle vortexing, or by pipetting up and down a few times.

Table 2. First-Strand cDNA Synthesis Reaction Set-up for RNA standards

Component	Volume per Reaction
Nuclease-Free Water	3.5 µL
2x microScript Reverse Transcription Master Mix	5 µL
microscript microRNA Enzyme Mix	0.5 µL
RNA Standard	1 µL
Total Volume	**10 µL**

* If a sub-pg per µL RNA sample is suspected, prepare an additional RNA standard concentration of 500 fg per µL by mixing 5 µL of the 1 pg per µL stock with 5 µL of nuclease-free water.

5. Incubate First-Strand cDNA Synthesis reaction in a thermocycler as described in **Table 3**.

Table 3. Reaction Protocol for First-Strand cDNA Synthesis

Temperature	Time
37°C	30 minutes
50°C	30 minutes
70°C	15 minutes
4°C	Hold

6. Dilute 2 µL of cDNA into 28 µL of nuclease-free water. Use the diluted cDNA for PCR-based quantification in Section C. Un-used and un-diluted cDNA can be stored at -20°C.

C. Quantitative PCR Assay Preparation

Notes Before Use:

- The Real-Time PCR Master Mix contains a fluorescent DNA dye compatible to SYBR Green/FAM detection.
- To avoid contamination, add the components to the PCR tubes in the order shown in the tables below (ie: 1) Nuclease-free water; 2) 2x PCR Master Mix; 3) Primer Set; and 4) the Sample DNA or Standards).

1. Thaw the 2x Real-Time PCR Master Mix on ice.

2. Prepare the PCR reaction as shown in Table 4 below. Mix the components by gentle vortexing, or by pipetting up and down a few times. Keep the reaction on ice and away for light prior to performing the Real-Time PCR.

Table 4. qPCR Assay Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	5.5 µL
2X Real-Time PCR Master Mix	10 µL
RNA Quantification Primer Set Mix	2 µL
Diluted cDNA (Standard or Sample)	2.5 µL
Total Volume	20 µL

D. PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 5 below. Set up plate read of SYBR Green/FAM dye for **Cycle 2, Step 3**.
2. Run the Real-Time PCR.

Table 5. PCR Assay Program

Real-Time PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (40x)	**Step 1**	**94°C**	**15 sec**
	Step 2	**60°C**	**30 sec**
	Step 3	**72°C**	**45 sec**

E. RNA Quantification Assay Interpretation

1. For real-time analysis, using the analysis software of the thermocycler, generate a standard cure using the Ct values of the RNA Standard dilution series. The standard curve can then be used to determine the starting quantity of the sample of interest.
2. If the amount of sample RNA and the amount of RNA Standard dilution series used per RT-PCR reaction are not the same, ensure that the proper volume adjustment is made in order to attain the correct RNA concentration of the sample. Two examples are illustrated below:

Example 1 – RNA Sample used in Step 3 (Table 1) is 1 µL and RNA Standard used is 1 µL

If observed sample RNA concentration based on Standard Curve is 50 pg/µL, then

$$\textbf{Sample RNA Concentration} = 50\ \text{pg/µL} \times \frac{\text{(Standard Volume, 1 µL)}}{\text{(Sample Volume, 1 µL)}} = 50\ \text{pg/µL}$$

Example 2 – RNA Sample used in Step 3 (Table 1) is 2.5 µL and RNA Standard used is 1 µL

If observed sample RNA concentration based on Standard Curve is 50 pg/µL, then

$$\textbf{Sample RNA Concentration} = 50\ \text{pg/µL} \times \frac{\text{(Standard Volume, 1 µL)}}{\text{(Sample Volume, 2.5 µL)}} = 20\ \text{pg/µL}$$

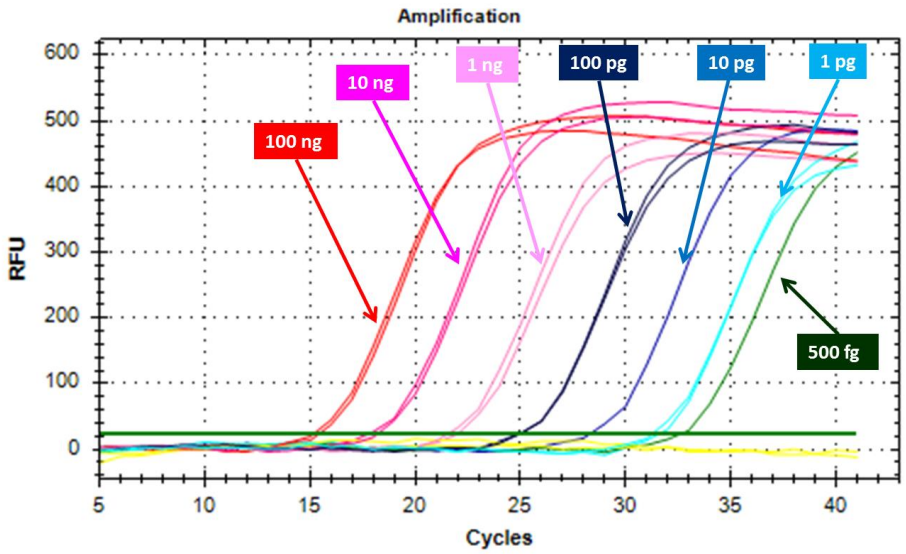

Figure 1: A representative RT-qPCR baseline graph showing the successful amplification of a dilution series of Quantified RNA Standard.

F. Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Related Products	Product #
Plasma/Serum RNA Purification Mini Kit	55000
Plasma/Serum Exosome Purification and RNA Isolation Mini Kit	58300
Plasma/Serum Exosome and Free-Circulating RNA Isolation Mini Kit	59500
Urine Cell-Free Circulating RNA Purification Mini Kit	56900
Urine Exosome Purification and RNA Isolation Mini Kit	58400
Urine Exosome and Free-Circulating RNA Isolation Mini Kit	59200

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