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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 cell-free urine, plasma/serum or exosomes may yield total RNA within the typical range of 1 - 100 pg/µL or lower. 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 ~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 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 over a wide dynamic range of concentrations, including mid pg per µL to sub-femtogram (fg) per µL. The kit enables two sequential enzymatic reactions – reverse transcription; and Poly(A) tailing using Norgen's microScript Reverse Transcription system followed by Real-Time PCR to amplify human RNA/cDNA from different types of inputs (such as various liquid biopsies). The unknown sample 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	20 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 Standard 50 pg/µL	2 x 60 µL*
Nuclease-Free Water	3 x 1.25 mL
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* The concentrated standard is provided in two tubes to reduce the number of freeze/thaw cycles.

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

- Real-time PCR platform with FAM/Sybr Green channels
- 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
        - Plasma/Serum Exosome Purification and RNA Isolation Mini Kit Cat # 58300
        - Plasma/Serum Exosome and Free-Circulating RNA Isolation Mini Kit Cat # 59500
        - Urine Cell-Free Circulating RNA Purification Mini Kit Cat # 56900
        - Urine Exosome Purification and RNA Isolation Mini Kit Cat # 58400
        - Urine Exosome and Free-Circulating RNA Isolation Mini Kit Cat # 59200
        - Cell Culture Media Exosome Purification and RNA Isolation Mini Kit Cat # 60700
- 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

1. 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 listed above. It is highly recommended to perform a DNase treatment on the sample to maximize RNA quantification precision.

2. 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 fresh 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 RNA elution.**

B. Standard Preparation

1. From the provided 50 pg/uL RNA Standard, perform six 1:10 serial dilutions as outlined in Table 1 below using the provided nuclease-free water to generate 5 pg/µL, 0.5 pg/µL, 50 fg/µL, 5 fg/µL, 0.5 fg/µL, and 50 ag/µL RNA Standards.

Table 1. Standard Preparation

Quantified RNA Standard	Volume of Nuclease-Free Water	Volume of Standard of Different Concentration
50 pg/µL	Original Standard No dilution Required	Original Standard No dilution Required
5 pg/µL	18 µL	2 µL of Standard (50 pg/µL)
0.5 pg/µL	18 µL	2 µL of Standard (5 pg/µL)
50 fg/µL	18 µL	2 µL of Standard (0.5 pg/µL)
5 fg/µL	18 µL	2 µL of Standard (50 fg/µL)
0.5 fg/µL	18 µL	2 µL of Standard (5 fg/µL)
50 ag/µL	18 µL	2 µL of Standard (0.5 fg/µL)

C. Procedure for First-Strand microRNA cDNA Synthesis

- The amount of 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 2 and 3 below to prepare the Sample Assay and Standard Curve
    1. Prepare the First-Strand cDNA Synthesis Reaction mixture for the Sample RNA (Table 2)
    2. Prepare the First-Strand cDNA Synthesis Reaction for the RNA Standards (Table 3)
- 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. This kit and procedure could be used to quantify as little as 100 ag/µL of 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 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 samples

Component	Volume per Reaction
Nuclease-Free Water	2.9 µL
2x microScript Reverse Transcription Master Mix	5 µL
microscript microRNA Enzyme Mix	0.1 µL
RNA to be quantified*	2 µL
Total Volume	**10 µL**

* Between 1 to 4.9 µL of RNA sample could be used per assay. If a volume other than 2 µL RNA is to be used, maintain the 10 µL reaction volume by adjusting 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 3**. Mix the components by gentle vortexing, or by pipetting up and down a few times.

Table 3. First-Strand cDNA Synthesis Reaction Set-up for RNA standards

Component	Volume per Reaction
Nuclease-Free Water	2.9 µL
2x microScript Reverse Transcription Master Mix	5 µL
microscript microRNA Enzyme Mix	0.1µL
RNA Standard	2 µL
Total Volume	**10 µL**

5. Perform First-Strand cDNA Synthesis in a thermocycler as described in **Table 4**.

Table 4. Reaction Protocol for First-Strand cDNA Synthesis

Temperature	Time
37ºC	30 minutes
50ºC	30 minutes
70ºC	15 minutes
4ºC	Hold

6. . Use the cDNA for PCR-based quantification in Section C. Un-used cDNA can be stored at -20ºC.

D. Quantitative PCR Assay Preparation

Notes Before Use:

- The Real-Time PCR Master Mix contains a fluorescent DNA dye compatible with 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 5 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 5. 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
cDNA (Standard or Sample)	2.5 µL
Total Volume	20 µL

E. PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 6 below. Set up plate read of SYBR Green/FAM dye for **Cycle 2, Step 3**.
2. Run the Real-Time PCR.

Table 6. 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**

F. RNA Quantification Assay Interpretation

1. For real-time analysis, using the analysis software of the thermocycler, generate a standard curve 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. Divide the amount of sample RNA by the volume of sample RNA input in µL.

Table 7. Standard Curve generation

Quantified RNA Standard	amount in 20 µL reaction spiked with 2 µL of standard
50 pg/µL	100 pg
5 pg/µL	10 pg
0.5 pg/µL	1 pg
50 fg/µL	100 fg
5 fg/µL	10 fg
0.5 fg/µL	1 fg
50 ag/µL	100 ag

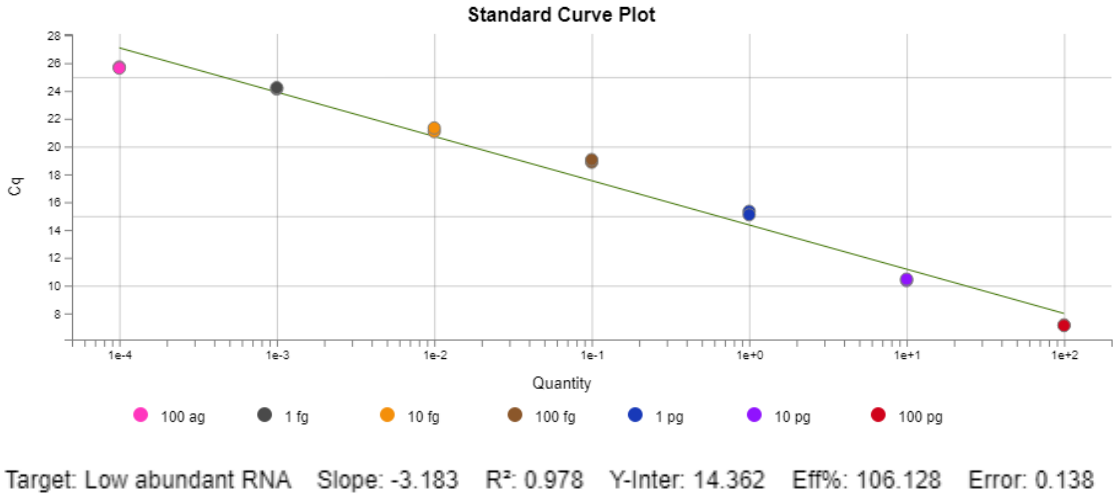

Figure 1: A representative Standard Curve showing the successful amplification of a dilution series of Quantified RNA Standard.

Example calculation to determine unknown RNA concentration of a sample:

Using Y= mx+b
solve for x by: x= (Y-b)/m
where m= slope b= Y-intercept, and Y= the Cq value.

G. 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
Cell Culture Media Exosome Purification and RNA Isolation Mini Kit	60700

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