NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

NGS Library Quantification Kit (for Small RNA-Seq) Product Insert

Product # 61600

Next Generation Sequencing (NGS) has become a widely adopted technology for studying many aspects of molecular biology including genome and exome sequencing, targeted sequencing and metagenomics, as well as for various RNA sequencing including mRNA-Seq and small RNA-Seq. An NGS library is usually generated by adding an adapter (with ID index) to a DNA or cDNA library using different technologies including ligation and PCR amplification. Accurate quantification of NGS libraries is essential for maximizing data output and quality from each sequencing run. In particular, it is important to (1) ensure that the adapter-index is properly incorporated; (2) properly quantify each individually-indexed library for equal representation during library pooling; (3) accurately quantify the final library pool for optimal cluster generation. Poor library quantification due to under-estimation or over-estimation may lead to over-clustering or inability to maximize the sequencing output capacity, respectively. The use of qPCR with sequence-specific primers provides a more accurate nucleic acid measurement than standard techniques such as spectrophotometry, fluorescent dye or capillary electrophoresis as only the adapter-ligated molecules are quantified.

Norgen's NGS Library Quantification Kit (for Small RNA-Seq) offers a PCR-based detection procedure to quantify NGS libraries (specifically Small RNA-Seq) of a wide spectrum of concentrations. The kit consists of a specially designed primer mix compatible with the Illumina system, that is used in conjunction with the provided 2x Real-Time PCR Master Mix to amplify a library of unknown concentration. The unknown library is accurately quantified using a standard curve constructed from the provided DNA Standard (range from 20 pM to 2 fM) on a Real-Time PCR System. The kit is specially optimized to quantify Small RNA-Seq libraries with DNA standards that have similar sizes to a Small RNA-Seq library. However, it could also be used with other types of NGS libraries.

Specifications

Component	Product # 61600 (100 reactions)
2x Real-Time PCR Master Mix	1 mL
NGS Library Quantification Primer Set Mix	600 µL
Quantified NGS Library Standards (for Small RNA-Seq)[1]	5 Standards, each 80 µL
Product Insert	1

[1]Quantified Library Standards are provided as 20 pM, 2 pM, 200 fM, 20 fM and 2 fM

Storage Conditions

Upon receipt, store Norgen's NGS Library Quantification Kit (for Small RNA-Seq) 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 NGS Library Quantification Kit (for Small RNA-Seq) is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

Norgen's NGS Library Quantification Kit (for Small RNA-Seq) 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
- Disposable powder-free gloves
- Benchtop microcentrifuge
- Micropipettors
- Sterile pipette tips with filters
- Nuclease-Free Water
- PCR tubes

Warnings and Precautions

- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling 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 samples 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 substitute or mix reagents from different kit lots or from other manufacturers.
- 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

Notes Before Use:

- Before use, suitable amounts of all PCR components should be completely thawed at room temperature, mixed by gentle vortexing or by pipetting, and centrifuged briefly. Put all thawed components on ice.
- Avoid repeated freeze and thaw. If needed, make small aliquots of each PCR component and store at -20^{o}C or lower.
- Work quickly on ice

1. Pipette the entire 600 µL **NGS Library Quantification Primer Set Mix** into the tube containing the **2x Real-Time PCR Master Mix**.

2. The Master Mix with Primers can be stored as is. However, it is recommend to make small aliquots and store un-used portions at -20^{o}C or lower to avoid multiple freeze-thaw cycles.

B. NGS Library Dilution

1. Prepare a 1:1,000 dilution of the library of interest. Mix 1 µL of the library to be quantified with 999 µL of nuclease-free water (provided by user).

2. Prepare a **1:10,000 dilution** of the library of interest. Mix 10 µL of the 1:1,000 dilution with 90 µL of nuclease-free water (provided by user).

3. Prepare a **1:100,000 dilution** of the library of interest. Mix 10 µL of the 1:10,000 dilution with 90 µL of nuclease-free water (provided by user).

4. In general, we recommend using the **1:10,000 dilution** and **1:100,000 dilution** of the library for qPCR quantification.

> **Note**: Most NGS Library Preparation workflows yield a final library concentration of 1 to 200 nM. For 1:10,000 dilution, it will result in a range of 100 fM to 20 pM. For 1:100,000 dilution, it will result in a range of 10 fM to 2 pM. This will ensure the diluted library falls within the range of the provide Library Standard Curve of 2 fM to 20 pM. If a lower concentration of library is expected, the 1:1,000 dilution may be used instead.

C. NGS Library Quantification PCR Assay Preparation

Notes Before Use:

- Work quickly on ice.
- The amount of **2x Real-Time PCR Master Mix** and **NGS Library Quantification Primer Set Mix** provided is enough for up to 100 qPCR reactions
- Follow the order outlined in Tables 1 to 3 below to prepare the No Template Control, Sample Assay and Standard Curve
    1. Prepare the No Template Control PCR (Table 1)
    2. Prepare the Sample Assay PCR (Table 2)
    3. Prepare Standard Dilution Series PCR (Table 3)
- It is recommended to set up triplicates for both PCR Standards and library samples to achieve best accuracy.

1. Prepare the PCR reaction for No Template Control as shown in Table 1 below. It is highly recommended to include a No Template Control in the PCR. The C_t values obtained will not be used in the standard curve but it could provide an indication if there is sample contamination in the assay.

Table 1. No Template Control PCR Sample Assay Preparation

PCR Components	Volume Per PCR Reaction
Nuclease-Free Water	4 µL
2X Real-Time PCR Master Mix with NGS Library Quantification Primer Set	16 µL
Total Volume	20 µL

2. Prepare the PCR reaction for sample library dilutions as shown in Table 2 below. Use of both 1:10,000 dilution and 1:100,000 dilution (prepared in **Section B**) is highly recommended.

Table 2. Library Sample Assay Preparation

PCR Components	Volume Per PCR Reaction
Diluted Library	4 µL
2X Real-Time PCR Master Mix with NGS Library Quantification Primer Set	16 µL
Total Volume	20 µL

3. Prepare the PCR reaction for Library Standards as shown in Table 3 below. Prepare reactions for each of the 5 **Quantified NGS Library Standards** (20 pM, 2 pM, 200 fM, 20 fM and 2 fM) provided.

Table 3. Library Standard Assay Preparation

PCR Components	Volume Per PCR Reaction
Quantified NGS Library Standard	4 µL
2X Real-Time PCR Master Mix with NGS Library Quantification Primer Set	16 µL
Total Volume	20 µL

D. PCR Assay Programming

1. Program the thermocylcer according to the program shown in Table 3 below.
2. Run the Real-Time PCR.

Table 3. PCR Assay Program

Real-Time PCR Cycle	Step	Temperature	Duration
Cycle 1	**Step 1**	**95°C**	**3 min**
Cycle 2 (35x)	**Step 1**	**94°C**	**30 sec**
	Step 2	**62°C**	**45 sec**

E. DNA Quantification Assay Interpretation

1. For real-time analysis, use the analysis software of the thermocycler to generate a standard curve using the C_t values of the DNA Standard dilution series. Confirm the PCR efficiency is around 90% or higher. The standard curve can then be used to determine the starting quantity of the sample of interest.

2. Calculate the actual concentration of the library by adjusting with the dilution factor.

Example 1 – Library sample was diluted as 1:10,000

If observed sample library concentration based on Standard Curve is 5 pM, then

Sample Library Concentration = 5 pM x Dilution Factor (10,000) = 50 nM

3. If the average library size is different than that of the **Library Standard** (140 bp), also adjust the concentration accordingly as follows:

Example 2 – Library sample was diluted as 1:10,000 with average size of 400 bp.

If observed sample library concentration based on Standard Curve is 5 pM, then

Sample Library Concentration

$$= 5\text{ pM} \times \text{Dilution Factor (10,000)} \times \frac{140}{\text{Library Size (400)}} = 17.5\text{ nM}$$

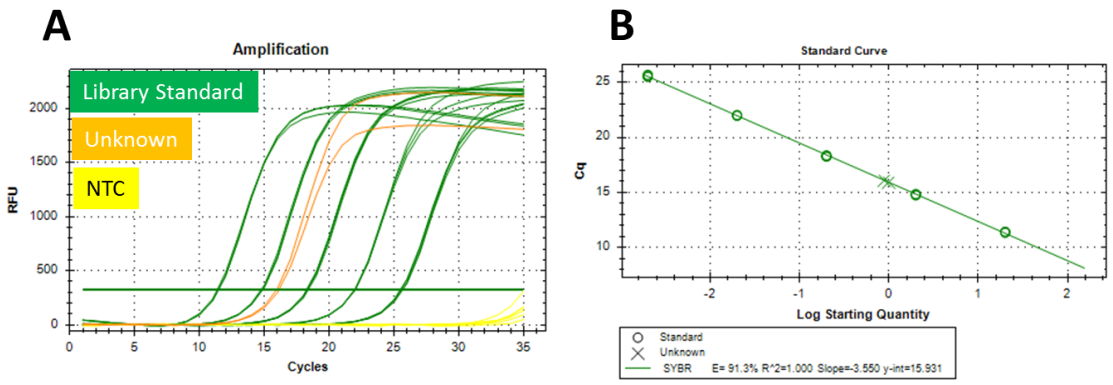

Figure 1: A representative qPCR baseline graph showing the successful amplification of Quantified NGS Library Standards (Green) with a range from 20 pM to 2 fM, using Norgen's NGS Library Quantification Kit (for Small RNA-Seq) (Panel A). Duplicate amplification of a sample Small RNA-Seq library (at 1:10,000 dilution) was performed (Orange). The derived library concentration was 9.41 nM. Norgen's NGS Library Quantification Kit (for Small RNA-Seq) is of good quality as shown with the high PCR efficiency and correlation in the standard curve (Panel B) with low background signals (No Template Control – NTC as Yellow in Panel A).

Related Products	Catalogue Number
PCR Purification Kit	14400, 24800, 45700
PCR and Sequencing Reaction Clean-Up Kit (Magnetic Bead System)	60200
Size-Select Kit for NGS Library Preparation	53600
CleanAll RNA/DNA Clean-Up and Concentration Kit	23800

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.

Norgen Biotek Corp.
3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

