PCRBIOSYSTEMS
simplifying research

www.pcrbio.com

NGSBIO Library Quant Kit for Illumina® Separate-ROX

Product description

The NGSBIO Library Quant Kit uses qPCR to quantify adapter-ligated molecules for use in all Illumina® Next Generation Sequencing (NGS) systems. qPCR is the best method for quantifying NGS libraries because it only measures the molecules that can be used as templates for library amplification and cluster generation. The 5 DNA standards provided in the kit are precisely measured to allow easy and accurate quantification.

The kit includes qPCRBIO SyGreen Mix, which uses antibody-mediated hot start to ensure all reactions start simultaneously. The advanced buffer system has been developed using our high-throughput smart screen technology to ensure efficient amplification from both GC-rich and AT-rich templates. The NGSBIO Library Quant Kit gives consistent and reliable quantification across a wide range of sample types, concentrations, fragment sizes (up to 1000bp) and GC content.

Library quantification can be used at any time after adapter ligation and should always be used prior to cluster generation. Overestimating library concentration can result in insufficient cluster density and underestimating library concentration can result in a high, saturating cluster density. To learn more about optimal cluster density, consult the instructions of your Illumina® machine.

For data analysis we recommend using the NGSBIO Library Quantification Tool available at https://pcrbio.com/resources/ngsbio-tool/.

For more detailed instructions, users can download a full technical manual at https://pcrbio.com/products/pcr/ngsbio-library-quant-kit/#tab-documents.

Kit contents

Component	100 Rxns	500 Rxns
2x qPCRBIO SyGreen Mix No-ROX	1 x 1mL	5 x 1mL
50µM ROX Additive	1 x 200µL	1 x 200µL
DNA Standards 1-5	30µL each	85µL each
10x Illumina® Primers	1 x 1mL	1 x 0.2mL
10x Dilution Buffer	2 x 1.5mL	1 x 0.6mL

Shipping and storage

On arrival the kit should be stored between -30°C and -15°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

Limitations of product use

The product may be used for in vitro research purposes only.

Technical support

Help is available on our website at https://pcrbio.com/resources/ including answers to frequently asked technical questions. For technical support and troubleshooting you can submit a technical enquiry online or email technical@pcrbio.com with the following information:

- Average library molecule length
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile

Important considerations

NGS instrument compatibility: This kit is compatible with the Illumina® iSeq, MiniSeq, MiSeq, NextSeq, HiSeq, HiSeq X, and NovaSeq instruments.

PCR instrument compatibility: Different real-time PCR instruments require different levels of ROX passive reference. Please check our qPCRBIO Selection Tool to determine which ROX concentration your instrument requires (https://pcrbio.com/resources/qpcr-selection-tool/).

ROX Additive protocol: Use the following chart to add the correct amount of 50µM ROX Additive directly to the 1mL tube of 2x qPCRBIO master mix supplied. Vortex thoroughly after ROX addition. Once the ROX is added, the reagent may be used straight away or stored between -30˚C and -15˚C for future use.

Reagent	Hi-ROX instruments	Lo-ROX Instruments
2x qPCRBIO SyGreen Mix No-ROX	1.0mL	1.0mL
50µM ROX Additive	20.0µL	2.0µL

Reaction setup

1. Before starting, briefly vortex and spin down the 2x qPCRBIO SyGreen Mix, DNA Standards, and 10x Illumina® Primers.
2. Add 1 part 10x Dilution Buffer to 9 parts water and mix thoroughly. Use this mixture to dilute libraries 10^6x. PCR grade water or a weak buffer, such as 10 mM Tris pH 8.0, may be used instead.
3. Prepare a master mix based on the following table. It is recommended to make enough for three replicates of each standard and each library sample:

Reagent	20µL reaction	Final concentration	Notes
2x qPCRBIO SyGreen Mix	10µL	1x	
10x Illumina® Primers	2.0µL	400nM each P5 and P7	P5: 5'-AAT GAT ACG GCG ACC ACC GA-3' P7: 5'-CAA GCA GAA GAC GGC ATA CGA-3'
Diluted sample or DNA Standard	4.0µL	variable	
PCR grade dH_2O	4.0µL		

4. Program the instrument using the following conditions, acquiring data on the FAM channel:

Cycles	Temperature	Time	Notes
1	95˚C	1 minute	Polymerase activation
40	95˚C 63˚C	15 seconds 45 seconds	Denaturation Anneal/extension
Melt analysis	Refer to instrument instructions		Optional melt profile analysis

Analysis

5. Create a standard curve using the Ct values of the DNA standards and calculate the efficiency. Efficiency should be between 90% - 110% for accurate quantification. The standards range from 2pM to 0.2fM and only samples that fall within this range can be accurately quantified.
6. Calculate the concentration of each sample dilution using the standard curve, then calculate the concentration of the undiluted sample and adjust for size:

$$\text{library concentration} = \text{reaction concentration} \times \text{dilution factor} \times \frac{452}{\text{average fragment length}}$$

For fast and accurate calculation we recommend using the NGSBIO Library Quantification Tool available at https://pcrbio.com/resources/ngsbio-tool/.