



PCRBIO SYSTEMS
simplifying research

NGSBIO Library Quant Kit for Illumina® Separate-ROX

www.pcrbio.com

Product description

The NGSBIO Library Quant Kit uses qPCR to quantify adapter-ligated molecules for use in all Illumina Next Generation Sequencing (NGS) systems. 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 NGSBIO Library Quant Kit gives consistent and reliable quantification across a wide range of sample types, concentrations, fragment sizes (up to 1000 bp) and GC content. Library quantification can be used at any time after adapter ligation and should always be used prior to cluster generation. 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/>.

Quality control

PCR Biosystems operates under an ISO 13485 certified Quality Management System. Our products are extensively tested and undergo a comprehensive, multi-step quality control process according to ISO 13485 standards, to ensure optimum performance, consistency and traceability.

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

Shipping and storage

On arrival the kit should be stored between -30 °C and -20 °C. Avoid prolonged exposure to light. If stored correctly, the kit will retain full activity until the indicated expiry date. The kit can be stored at 4 °C for 1 month.

Technical support

Scan or click the QR code for troubleshooting help and answers to frequently asked technical questions. For further technical support, please email technical@pcrbio.com with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of amplification traces and melting profile



TROUBLESHOOT



FAQS

Product Use: Unless we agree otherwise in writing, the Goods we supply are provided:

1. For research purposes only and you should not use or rely on the Goods for diagnostic purposes. If you wish to use the Goods in a regulatory approved medical device, please contact us so that we may consider this and discuss it further with you.
2. Subject to our standard terms and conditions found at <https://pcrbio.com/terms-conditions/>.

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 qPCR BIO 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 50 µM ROX Additive directly to the 1 mL tube of 2x qPCR BIO 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 -20 °C for future use.

Reagent	Hi-ROX instruments	Lo-ROX Instruments
2x qPCR BIO SyGreen® Mix No-ROX	1 mL	1 mL
50 µM ROX Additive	20 µL	2 µL

Reaction setup

- Before starting, briefly vortex and spin down the 2x qPCR BIO SyGreen® Mix, DNA Standards, and 10x Illumina® Primers.
- Add 1 part 10x Dilution Buffer to 9 parts water and mix thoroughly. Use this mixture to dilute libraries 10⁶x. PCR grade water or a weak buffer, such as 10 mM Tris pH 8.0, may be used instead.
- 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	20 µL reaction	Final concentration	Notes
2x qPCR BIO SyGreen® Mix	10 µL	1x	
10x Illumina® Primers	2 µL	400 nM 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 µL	variable	
PCR grade dH ₂ O	4 µL		

- Program the instrument using 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

- 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.
- Calculate the concentration of each sample dilution using the standard curve, then calculate the concentration of the undiluted sample and adjust for size:

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

For fast and accurate calculation we recommend using the NGS BIO Library Quantification Tool available on our website at <https://pcrbio.com/resources/ngsbio-tool/>.

