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NGSBIO Library Quant Kit Blue for Illumina® Lo-ROX

Product description

The NGSBIO Library Quant Kit Blue 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. A non-reactive blue dye has been added to assist researchers during pipetting.

The kit includes qPCRBIO SyGreen® Blue 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/

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

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® Blue Mix Lo-ROX	1 x 1 mL	5 x 1 mL
DNA Standards 1-5	30 µL each	85 µL each
10x Illumina® Primers	1 x 0.2 mL	1 x 1 mL
10x Dilution Buffer	1 x 0.6 mL	2 x 1.5 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

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/).

Reaction setup

1. Before starting, briefly vortex and spin down the 2x qPCRBIO SyGreen® Blue 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	20 µL reaction	Final concentration	Notes
2x qPCRBIO SyGreen® Blue 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_2O	4 µL		

4. 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

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 on our website at https://pcrbio.com/resources/ngsbio-tool/.