

PerfeCta[®] NGS Quantification Kit

Real-Time qPCR is the most sensitive and precise method for quantifying adapter-ligated DNA molecules. PerfeCta NGS Quantification kits provide a comprehensive reagent solution in a single easy-to-use kit.

FEATURES & BENEFITS:

- Precise quantification of adapter-tagged library molecules
- Accurate and sensitive method for NGS library quantification
- Stabilized, prediluted standards for convenient use
- Consistency across a broad range of samples

DESCRIPTION:

Accurate quantification of the number of amplifiable library molecules is the most critical step in the NGS workflow in obtaining high quality read data with next-generation sequencing technologies. The PerfeCta NGS Quantification Kit uses real-time PCR to specifically quantify library molecules that possess the appropriate adapter tag at each end. These are the suitable template molecules for Bridge PCR used for Illumina NGS platforms. PerfeCta NGS Quantification Kits simplify the library quantification process by providing stabilized, pre-diluted standards, pre-qualified primer sets, and an optimized dilution buffer for your NGS library samples. This minimizes pipetting errors and ensures reproducible and precise qPCR results, even with dilute samples. The robust qPCR performance of PerfeCta SYBR[®] Green SuperMix provides accurate quantification of NGS libraries with varying fragment sizes or GC content.

A common problem with some NGS library quantification protocols is the use of DNA standards that are too concentrated and generate qPCR data that are outside of the linear dynamic range for many qPCR instruments. Improper baseline settings result in compressions between the highest concentrated DNA standards that in turn give rise to inflated PCR efficiencies and inaccurate library quantification results.

The NGS DNA standards supplied with the PerfeCta NGS Quantification Kits have been carefully selected to avoid these artifacts and produce NGS library standard curves with exceptionally high linear regression correlation coefficients.

Illumina

The DNA standard for Illumina NGS platforms generates a 426 bp amplicon (48.8% GC). Primer sequences correspond to the "P5" and "P7" primer sequences for Illumina sequencing libraries:

Illumina forward primer: 5'-AAT GAT ACG GCG ACC ACC GA-3'

Illumina reverse primer: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

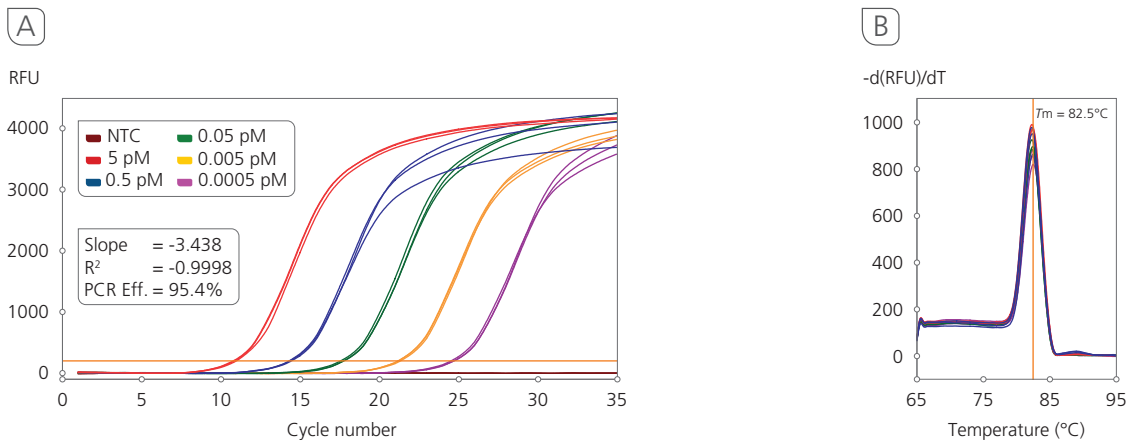


Figure 1 PerfeCta NGS Quantification Kit performance data. qPCR amplification of each of the five supplied DNA standards for Illumina NGS libraries (panel A) were carried out with the supplied primer sets (300 nM final concentration) and PerfeCta SYBR Green SuperMix in 20 μ l reaction volumes on a Bio-Rad CFX-96. Reactions were incubated for 5 min at 95°C followed by 35 cycles of: 95°C, 10 s; 60°C, 20 s; 45 s, 72°C. Real-time fluorescence data was collected and analyzed at completion of the 72°C extension step. After completion of PCR, a dissociation (melt) curve was performed to verify amplification of a single specific product (panels B).

ORDER INFO

Product Name	Quantabio Catalog Number	Size
PerfeCta NGS Quantification Kit - Illumina - 500 R	95154-500	500 x 20 μ l rxns
PerfeCta NGS Quantification Kit - Illumina, ROX - 500 R	95155-500	500 x 20 μ l rxns
PerfeCta NGS Quantification Kit - Illumina, Low ROX - 500 R	95156-500	500 x 20 μ l rxns

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Quantabio products are intended for molecular biology applications. The products are not intended for the diagnosis, prevention or treatment of a disease.

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