Quick-Start Protocol September 2017

QIAseq™ CleanStart PCR Kit

The QIAseq CleanStart PCR Kit (cat. no. 180795) should be stored immediately upon receipt at –30°C to –15°C. If stored under these conditions, the kit contents are stable until the date indicated on the QC label.

Further information

- *QIAseq CleanStart PCR Handbook*: **www.qiagen.com/HB-2463**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- QIAseq CleanStart PCR reagents use a proprietary amplification reaction, in conjunction with modification enzymes, to ensure that previously constructed NGS libraries are removed. If a previously amplified CleanStart library needs to be re-amplified, for instance, if an additional library is needed to replace a failed NGS run, omit the decontamination step of the PCR protocol to disable selective degradation.
- QIAseq CleanStart PCR reagents are included in several QIAseq NGS kits and are compatible with any standard, Illumina-compatible NGS libraries. These reagents are not compatible with libraries made for other NGS platforms, and may not be compatible with libraries utilizing custom adapters and sequencing primers (see kit handbook).

CleanStart amplification

1. Before starting, thaw CleanStart PCR Mix, 2X and the CleanStart PCR Primer Mix on ice. Mix thoroughly. Template libraries should be in a total volume of 23.5 µl. If template volume is less, adjust to 23.5 µl with sterile, DNA-free water.

2. Add 1.5 µl CleanStart PCR Primer Mix and 25 µl CleanStart PCR Master Mix, 2X to each sample and perform amplification as described in Table 1.

Table 1. CleanStart library amplification conditions

Step	Time	Temperature	Number of cycles
CleanStart decontamination*	15 min	37°C	1
Initial denaturation	2 min	98°C	1
PCR	20 s 30 s 30 s	98°C 60°C 72°C	Variable†
Final extension	1 min	72°C	1
Hold	∞	4°C	Hold

* For the re-amplification of libraries, omit the CleanStart decontamination step and start with incubation at 98°C for 2 min.

† Follow the recommendation of the NGS kit provider for the appropriate number of cycles. This will depend on the kit used, the amount of input material and other factors, but is usually in the range of 8–16 cycles for most applications.

Amplified libraries can be stored –20°C in a constant-temperature freezer for prolonged periods. Please note that after PCR, these libraries will still contain excess primers, which will interfere with sequencing. We recommend purifying libraries according to the NGS kit provider's specifications.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.