Quick-Start Protocol September 2016

QIAseq cfDNA Library T Kit

Upon receipt, store the QIAseq cfDNA Library T Kit (24) at –30°C to –15°C. This protocol is for the preparation of DNA libraries from circulating cell-free DNA (cfDNA) for next-generation sequencing with Ion Torrent™ instruments.

Further information

- *QIAseq cfDNA All-in-One T Kit Handbook*: **www.qiagen.com/HB-2199**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Quantitate purified double-stranded cfDNA using a fluorometric method. Start with 1–100 ng cfDNA in Buffer EB, nuclease-free H_2O or 10 mM Tris·HCl (pH 8.0).
- Only use the chemically modified library adapters supplied with the kit.
- The single-use 24-plex Ion Torrent Adapter plate contains equimolar mixes of universal and barcode adapters.
- Refer to the kit handbook for equipment and reagents required.

End-polishing, adapter ligation and nick repair

1. Before setting up the All-in-One library preparation reaction mix, vortex and spin down the thawed adapter plate.
2. Set up the All-in-One library preparation reaction mix on ice according to Table 1. Remove the protective adapter plate lid. Pierce the foil seal for each well of the adapter plate to be used right before pipetting.

Table 1. All-in-One Library preparation reaction setup

Component	Volume/reaction (µl)
Input DNA (cfDNA)	Variable
All-in-One Reaction Buffer, 4x	22.5
All-in-One Enzyme Mix	6
QIAseq Adapter Mix (24-plex plate)	5
Nuclease-free water	Variable
Total reaction volume	**90 µl**

3. Mix by gently pipetting 5–6 times.
4. Load onto the thermal cycler and start the All-in-One reaction program (Table 2).
   IMPORTANT: Do not use a thermal cycler with a heated lid.

Table 2. End-polishing, adapter ligation and nick repair thermal cycling conditions

Program	Temperature	Time	Additional comments
End-polishing/Ligation	25°C	30 min	Polishing the ends of DNA fragments and adapter ligation
Nick repair	72°C	5 min	Inactivation of end-polishing and ligation enzymes; nick repair
	4°C	∞	Hold

5. When the program is complete, proceed immediately to cleanup.
6. Transfer 90 µl ligation reaction to a 1.5 ml LoBind tube or PCR plate.
7. Add 27 µl resuspended Agencourt® AMPure® XP beads to each sample and mix.
8. Incubate for 5 min at room temperature.
9. Pellet the beads on a magnetic stand and wait until the solution is clear.
10. Transfer 110 µl supernatant to a new tube or PCR plate. Discard the beads.
11. Add 27 µl resuspended Agencourt AMPure XP beads to each sample and mix.
12. Incubate for 5 min at room temperature.
13. Pellet the beads on a magnetic stand and wait until the solution is clear, then carefully discard the supernatant.

14. Add 200 µl fresh 80% ethanol to each pellet. Pellet the beads on the magnetic stand and carefully discard the supernatant.

15. Repeat step 14 for a total of 2 ethanol washes. Remove excess ethanol.

16. Incubate on the magnetic stand for 5–10 min or until the beads are dry. Over-drying may result in lower DNA recovery. Remove from the magnetic stand.

17. Elute by resuspending in 26 µl Buffer EB. Pellet beads on the magnetic stand. Carefully transfer 23.5 µl supernatant into a new PCR plate.

Library amplification

18. Program a thermal cycler with a heated lid according to Table 3.

Table 3. Library amplification cycling conditions

Incubation time	Temperature	Number of cycles
2 min	98°C	1
20 s 30 s 30 s	98°C 60°C 72°C	**Variable, depending on DNA input***: **7** (100 ng input); **10** (10 ng input); **12** (1 ng input)
1 min	72°C	1
∞	4°C	Hold

* Number of PCR cycles depends on input DNA amount and quality.

19. Prepare reactions on ice according to Table 4. Pipet 6–8 times to mix.

Table 4. Amplification reaction setup

Component	Volume/reaction (µl)
Library DNA	23.5
HiFi PCR Master Mix, 2x	25
Primer Mix	1.5
Total reaction volume	**50 µl**

20. Transfer the PCR plate to the thermal cycler and start the program.

21. When the program is complete, remove the plate and add 50 µl of resuspended Agencourt AMPure XP beads to each amplified library.
22. Follow steps 12–16 above. Elute by resuspending in 27 µl nuclease-free water or Buffer EB. Pellet the beads on the magnetic stand. Carefully transfer 25 µl supernatant to new tubes for storage.
23. Assess the library quality using a capillary electrophoresis method. The median library size will be the fragment size plus about 80 bp for the adapters. Libraries can be quantified with qPCR using a QIAseq Library Quant Array (sold separately).
24. Purified libraries can be stored at –20°C until ready for sequencing or hybrid capture.

Scan QR code for handbook.

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