Quick-Start Protocol September 2018

QIAseq® FastSelect™ RNA Removal Kit

QIAseq Stranded Total RNA Lib Kit RNA and/or Globin removal

Further information

- *QIAseq FastSelect RNA Removal Kit Handbook*: **www.qiagen.com/HB-2580**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000 or **www.qiagen.com/contact**

Notes before starting

- The QIAseq Stranded Total RNA Lib Kit (QIAGEN, cat. no. 180743, 180745) is required for use with this protocol.

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect RNA Removal Kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. To 100 ng – 1 µg of total RNA, add QIAseq FastSelect as follows:
   Option 1 (remove rRNA): Add 1 µl of rRNA Removal.
   Option 2 (remove Globin): Add 1 µl of Globin Removal.
   Option 3 (remove rRNA and Globin): Add 1 µl of rRNA Removal and 1 µl Globin Removal.
3. Bring the volume of the reaction to 29 µl.
4. From the QIAseq Stranded Total RNA Lib Kit, add 8 µl 5x RT Buffer to each sample. Briefly centrifuge, mix by pipetting up and down 10 times and centrifuge briefly again.
5. Incubate in a thermal cycler with a heated lid as described in according to your input RNA quality and approximate insert size.

IMPORTANT: Regardless of time and temperature chosen in Step 1, Steps 2-9 must be performed.

Table 1. QIAseq Stranded fragmentation and hybridization protocol

Input RNA quality	Step	Insert size ~150–250 bp	Insert size ~350 bp
High quality (RIN >9)	1*	15 min at 95°C	3 min at 95°C
Moderate quality (RIN 5–6)	1*	10 min at 95°C	3 min at 95°C
FFPE or degraded sample (RIN <3)	1*	No fragmentation†	No fragmentation†
Steps 2-9 are performed, regardless of Input RNA quality. They need to be performed whether the RNA is high quality, moderate quality, FFPE or degraded.	2	2 min at 75°C	2 min at 75°C
	3	2 min at 70°C	2 min at 70°C
	4	2 min at 65°C	2 min at 65°C
	5	2 min at 60°C	2 min at 60°C
	6	2 min at 55°C	2 min at 55°C
	7	5 min at 37°C	5 min at 37°C
	8	5 min at 25°C	5 min at 25°C
	9	Hold at 4°C	Hold at 4°C

* Choose one option for the Step 1 time, according to the input RNA quality and desired insert size.

† Also suitable for exosomal RNA or RNA of other origin with a size between 80–500 bp.

6. Refer to the *QIAseq Stranded Library Kit Handbook* and immediately perform Step 5 from "RNA fragmentation and reverse transcription."
   Note: Step 5 is specifically "After fragmentation, add 1 µl RT Enzyme, 1 µl RNase Inhibitor and 1 µl diluted DTT (0.4 M). Briefly centrifuge, mix by pipetting up and down 10 times and centrifuge briefly again."
7. Follow the *QIAseq Stranded Library Kit Handbook* to perform all remaining library construction steps.
   Note: When removing Globin, 2 additional cycles of library amplification may need to be performed.

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