June 2020

Quick-Start Protocol

QIAseq® FastSelect™ –rRNA Plant with the QIAseq Stranded Total RNA Lib Kit

The QIAseq FastSelect Kits for –rRNA Plant (cat. nos. 334315, 334317, 334319) may be used with the QIAseq Stranded Total RNA Lib Kit (cat. no. 180743 or 180745) to remove plant rRNA.

All components of QIAseq FastSelect should be stored in a constant-temperature freezer at –30 to –15°C. Under these conditions, the components are stable, without showing any reduction in performance and quality, until the date indicated on the box label.

Further information

- *QIAseq FastSelect –rRNA Plant Handbook*: **www.qiagen.com/HB-2783**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The QIAseq Stranded Total RNA Lib Kit is required for use with this protocol.
- Refer to the *QIAseq Stranded Total RNA Lib Kit Handbook* available at **www.qiagen.com/HB-2465**

Procedure

1. Thaw total RNA on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and return to ice.
2. Prepare the reagents required for the RNA fragmentation and QIAseq FastSelect –rRNA Plant.
   2a. Thaw 5x RT Buffer and nuclease-free water from the QIAseq Stranded Kit at room temperature (15–25°C).
   2b. Vortex 5x RT Buffer, nuclease-free water, and the tube(s) from the QIAseq FastSelect Kit, and then briefly centrifuge.
3. On ice, prepare the fragmentation/RNA removal reaction according to Table 1. Briefly centrifuge, mix by pipetting up and down 10 times, and centrifuge briefly again.
   Note: If setting up more than one reaction, prepare a volume of Master Mix that is 10% greater than what is required for the total number of reactions.

Table 1. Setup of fragmentation/RNA removal reactions

Component	Volume/reaction
Total RNA (100 ng – 1 µg)	Variable
RT Buffer, 5x*	8 µl
QIAseq FastSelect –rRNA Plant	1 µl
ERCC Control†	Optional
Nuclease-free water	Bring total reaction volume to 37 µl
Total volume	**37 µl**

* From the QIAseq Stranded Total RNA Lib Kit.

† ERCC Control RNA can be added according to the concentrations specified by the manufacturer. If added, the total fragmentation/RNA removal reaction volume should remain 37 µl.

4. Incubate as described in Table 2, according to your input RNA quality and desired insert size.

   Important: Regardless of time and temperature chosen in step 1, steps 2–9 must be performed.

Table 2. Combined QIAseq fragmentation and FastSelect 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	2 min at 37°C	2 min at 37°C
	8	2 min at 25°C	2 min at 25°C
	9	Hold at 4°C	Hold at 4°C

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

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

5. Refer to the *QIAseq Stranded Total RNA Lib Kit Handbook* and immediately proceed to "Protocol: First-strand Synthesis".
6. Follow the *QIAseq Stranded Total RNA Lib Kit Handbook* to perform all remaining library construction steps.

Revision History

Date	Changes
06/2020	Initial release

Scan QR code for handbook.

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