Quick-Start Protocol September 2018

QIAseq® FastSelect™ RNA Removal Kit

KAPA RNA HyperPrep Kit: rRNA 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 KAPA RNA HyperPrep Kit (Kapa Biosystems, cat. no. KK8540, KK8541) is required for use with this protocol.

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect RNA Removal Kit. Mix by vortexing and briefly centrifuge to collect residual liquid from the sides of the tubes.
2. From the KAPA RNA HyperPrep Kit, prepare the fragmentation and priming described in Table 1 at room temperature in a nuclease-free tube.

Table 1. KAPA RNA HyperPrep fragmentation and priming mix

Component	Volume/reaction
Total RNA (25 ng – 1 µg)	9 µl*
Fragment, Prime and Elute Buffer (2X) †	10 µl
Total volume	**19 µl**

* Reduce volume to 8 µl if removing rRNA and Globin.

† From KAPA RNA HyperPrep Kit.

3. To the assembled fragmentation and priming mix, 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.
4. Mix thoroughly by gently pipetting the reaction up and down several times and then briefly centrifuge to collect residual liquid from the sides of the tubes.
5. Incubate as in a thermal cycler with a heated lid described in Table 2, according to your input RNA quality.
   IMPORTANT: Regardless of time and temperature chosen in Step 1, Steps 2-9 must be performed.

Table 2. KAPA RNA HyperPrep fragmentation and hybridization protocol

Input RNA type	Step	Insert size ~350 bp
Intact	1*	**Choose:** 8 min at 94°C **or** 6 min 94°C **or** 6 min at 85°C
Partially degraded	1†	1 – 6 min at 85°C
Degraded (e.g. FFPE)	1‡	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
	3	2 min at 70°C
	4	2 min at 65°C
	5	2 min at 60°C
	6	2 min at 55°C
	7	5 min at 37°C
	8	5 min at 25°C
	9	Hold at 4°C

* Choose one option, depending if you want a desired mean library insert size of 100-200 bp (8 min at 94°C), 200-300 bp (6 min 94°C) or 300-400 bp (6 min at 85°C).

† For a desired mean library insert size of 100-300 bp.

‡ For a desired mean library insert size of 100-200 bp.

6. Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet* and immediately proceed to "1st Strand Synthesis" (Chapter 3 in v1.16 or v2.17).
7. Follow the *KAPA RNA HyperPrep Kit Technical Data Sheet* to perform all remaining library construction steps.
   Note: When removing Globin, 2 additional cycles of library amplification may need to be performed.