October 2019

Quick-Start Protocol

QIAseq® FastSelect™ –rRNA HMR and/or –Globin with the KAPA® RNA HyperPrep Kit

The QIAseq FastSelect Kits for –rRNA HMR (cat. nos. 334386, 334387, 334388), –Globin (cat. nos. 334376, 334377, 334378), and –rRNA/Globin (cat. nos. 335376, 335377, 335378) may be used with the KAPA RNA HyperPrep Kit (Roche, cat. no. KK8540 or KK8541) to remove human, mouse, or rat rRNA and/or globin.

All components of QIAseq FastSelect should be stored at –30 to –15°C in a constant-temperature freezer.

Further information

- *QIAseq FastSelect –rRNA HMR and –Globin Handbook*: **www.qiagen.com/HB-2670**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The KAPA RNA HyperPrep Kit is required for use with this protocol.
- Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet* (KR1350 – v2.17).

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. From the KAPA RNA HyperPrep Kit, prepare the fragmentation and priming mix 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 QIAseq FastSelect –rRNA HMR
   - **Option 2 (remove globin)**: Add 1 µl of QIAseq FastSelect –Globin
   - **Option 3 (remove rRNA and globin)**: Add 1 µl of QIAseq FastSelect –rRNA HMR and 1 µl of QIAseq FastSelect –Globin
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 in a thermal cycler with a heated lid as 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. Combined KAPA RNA HyperPrep fragmentation and FastSelect hybridization protocol

Input RNA type	Step	Time and temperature
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	2 min at 37°C
	8	2 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", section 3 in v2.17.
7. Follow the *KAPA RNA HyperPrep Kit Technical Data Sheet* to perform all remaining library construction steps.

   Important: It is highly recommended to dilute the KAPA adapters 1.5-fold compared to what is suggested in the default KAPA protocol.

   Important: When removing globin, 2 additional cycles of library amplification need to be performed.

Revision History

Date	Changes
10/2019	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.