October 2019

Quick-Start Protocol

QIAseq® FastSelect™ –Globin with the KAPA® mRNA HyperPrep Kit

The QIAseq FastSelect Kits for –Globin (cat. nos. 334376, 334377, 334378) and –rRNA/Globin (cat. nos. 335376, 335377, 335378) may be used with the KAPA mRNA HyperPrep Kit (Roche, cat. no. KK8580 or KK8581) to remove human, mouse, or rat 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 mRNA HyperPrep Kit is required for use with this protocol.
- Refer to the *KAPA mRNA HyperPrep Kit Technical Data Sheet* (KR1352 – v5.17).

Procedure

1. Follow the *KAPA mRNA HyperPrep Kit Technical Data Sheet*, "Library Construction Protocol", section 1 (Reagent Preparation).
2. Follow the *KAPA mRNA HyperPrep Kit Technical Data Sheet*, "Library Construction Protocol", section 2 (mRNA Capture).
3. Follow the *KAPA mRNA HyperPrep Kit Technical Data Sheet*, "Library Construction Protocol", section 3 (mRNA Elution, Fragmentation and Priming), steps 3.1–3.4.
4. Thaw the FastSelect –Globin tube from the QIAseq FastSelect kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
5. In place of step 3.5 in the *KAPA mRNA HyperPrep Kit Technical Data Sheet*, carefully transfer 19 µl of the supernatant containing the eluted, fragmented, and primed RNA into a separate plate or tube.
6. To the supernatant, add 1 µl of QIAseq FastSelect –Globin.
7. 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.
8. Incubate in a thermal cycler with a heated lid as described in Table 1, according to your input RNA quality.

Table 1. FastSelect hybridization protocol

Input RNA type	Step	Time and temperature
Hybridization	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

9. Refer to the *KAPA mRNA HyperPrep Kit Technical Data Sheet* and immediately perform step 3.6 ("Place the plate/tube[s] on ice and proceed immediately to **1st Strand Synthesis** [step 4]").
   Note: Step 3.6 is found in section 3 in KR1352 – v5.17.
10. Follow the *KAPA mRNA 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.