October 2019

Quick-Start Protocol

QIAseq® FastSelect™ –Globin with the TruSeq® Stranded mRNA Library Prep

The QIAseq FastSelect Kits for –Globin (cat. nos. 334376, 334377, 334378) and –rRNA/Globin (cat. nos. 335376, 335377, 335378) may be used with the TruSeq Stranded mRNA Library Prep (Illumina, cat. nos. 20020594 and 20020595) 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 TruSeq Stranded mRNA Library Prep is required for use with this protocol.
- Refer to the *TruSeq Stranded mRNA Reference Guide* (1000000040498).

Procedure

1. Using the *TruSeq Stranded mRNA Reference Guide*, purify mRNA as described under "Purify mRNA" steps 1–19 (pages 11–12 in 1000000040498).
2. Using the *TruSeq Stranded mRNA Reference Guide*, fragment mRNA as described under "Fragment mRNA" steps 1–15 (page 12 in 1000000040498).
3. Using the *TruSeq Stranded mRNA Reference Guide,* perform steps 1 and 2 under the "Procedure" section (page 13 in 1000000040498) of "Synthesize First Strand cDNA".
4. Thaw the QIAseq 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. To the 17 µl supernatant in the CDP plate, add 1 µl of QIAseq FastSelect –Globin.
6. Mix thoroughly by pipetting up and down several times and then briefly centrifuge to collect residual liquid from the sides of the tubes.
7. Incubate in a thermal cycler with a heated lid as described in Table 1.

Table 1. FastSelect hybridization protocol

Step	Time and temperature
1	2 min at 75°C
2	2 min at 70°C
3	2 min at 65°C
4	2 min at 60°C
5	2 min at 55°C
6	2 min at 37°C
7	2 min at 25°C
8	Hold at 4°C

8. Refer to the *TruSeq Stranded mRNA Reference Guide* and immediately proceed to and perform step 3 under the "Procedure" section (page 13) of "Synthesize First Strand cDNA".
9. Follow the *TruSeq Stranded mRNA Reference Guide* to perform all remaining library construction steps.

   Important: It is highly recommended to dilute the Illumina adapters 2-fold compared to what is suggested in the default Illumina 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.

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