October 2019

Quick-Start Protocol

QIAseq® FastSelect™ –Globin with the NEBNext® Poly(A) mRNA Magnetic Isolation Module & Ultra II Directional Library Prep 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 NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB, cat. no. E7490) and the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina® (NEB cat. nos. E7760S and E7760L) 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 NEBNext Poly(A) mRNA Magnetic Isolation Module and the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina are required for use with this protocol.
- Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* (Version 2.2).

Procedure

1. 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.
2. Referring to section 1 from the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual*, perform section 1.1 and section 1.2, steps 1.2.1 through 1.2.36 as indicated.
3. In place of steps 1.2.37 through 1.2.40, perform the following:
   - 3a. Incubate the sample in a thermal cycler (with the heated lid set at 105°C) for 15 min at 94°C *but do not cool the sample to 4°C*.
   - 3b. Immediately after the 94°C fragmentation has been completed, quickly spin down the tube in a microcentrifuge to collect the liquid from the sides of the tube and place on the magnet right away until the solution is clear (~1–2 min).
   - 3c. Collect the fragmented mRNA by transferring 10 µl of the supernatant to a nuclease-free 0.2 ml PCR tube.
   - 3d. Add 1 µl QIAseq FastSelect –Globin. Mix thoroughly by pipetting up and down several times and then briefly centrifuge to collect residual liquid from the sides of the tubes.
   - 3e. 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

4. Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* and immediately proceed to "First Strand cDNA Synthesis."
   Note: "First Strand cDNA Synthesis" is chapter 1.3 in Version 2.2 of the instruction manual.
5. Follow the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* to perform all remaining library construction steps.

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

Revision History

Date	Changes
10/2019	Initial release

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