Quick-Start Protocol September 2018

QIAseq® FastSelect™ RNA Removal Kit

TruSeq Stranded Library Prep 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 TruSeq Stranded mRNA Library Prep (Illumina, cat. no. 20020594, 20020595) is required for use with this protocol.

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect RNA Removal Kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. To 100 ng – 1 µg of total RNA, which is required to be in a maximum volume of 5 µl, 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.
3. From the TruSeq Stranded mRNA Library Prep, add 14.5 µl FPF (when using Option 1 or 2 above), or add 13.5 µl FPF (when using Option 3 above), to bring the volume of the reaction to 20.5 µl.

4. Mix thoroughly by pipetting up and down several times and then briefly centrifuge to collect residual liquid form the sides of the tubes.

   Incubate in a thermal cycler with a heated lid as described in Table 1.

   IMPORTANT: Irrespective of time at 94°C, steps 2-9 listed in Table 1 must be performed.

Table 1. TruSeq Stranded fragmentation and hybridization protocol

Step	Time and temperature
1*	8 min* at 94°C*
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

* The initial step at 94°C can be modified to permit longer RNA insert sizes. Please refer to Table 4 in the *QIAseq FastSelect RNA Removal Kit Handbook* for recommendations.

Note: The remaining steps 2-9 are performed regardless of the time at 94°C.

5. Use 17 µl of the fragmented/hybridized RNA, refer to the *TruSeq Stranded mRNA Reference Guide* and immediately proceed to "Synthesize First Strand cDNA."

   Note: From the *TruSeq Stranded mRNA Reference Guide*, the procedural step "Place the RBP plate on the magnetic stand and wait until the liquid is clear (~5 minutes)" is not applicable.

6. Follow the *TruSeq Stranded mRNA Reference Guide* to perform all remaining library construction steps.

   Note: When removing Globin, 2 additional cycles of library amplification may need to be performed.