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Quick-Start Protocol

QIAseq® FastSelect™ –Globin with the QIAseq Stranded mRNA Select 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 QIAseq Stranded mRNA Select Kit (cat. nos. 180773 and 180775) 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 QIAseq Stranded mRNA Select Kit is required for use with this protocol.
- Refer to the *QIAseq Stranded mRNA Select Kit Handbook* available at **www.qiagen.com/HB-2464**

Procedure

1. From the *QIAseq Stranded mRNA Select Kit Handbook*, perform "Protocol: mRNA Enrichment" with the recommended amount of total RNA input (100 ng – 1 µg). Ultimately elute the enriched mRNA in 27 µl.
2. Prepare the reagents required for the RNA fragmentation and QIAseq FastSelect –Globin removal.
   2a. Thaw 5x RT Buffer, nuclease-free water from the QIAseq Stranded kit, and the QIAseq FastSelect –Globin tube from the QIAseq FastSelect kit at room temperature.
   2b. Mix by vortexing and then briefly centrifuge.
3. On ice, prepare the fragmentation/RNA Removal reaction according to Table 1. Briefly centrifuge, mix by pipetting up and down 10 times, and centrifuge briefly again.
   Note: If setting up more than one reaction, prepare a volume of Master Mix that is 10% greater than what is required for the total number of reactions.

Table 1. Setup of fragmentation/RNA Removal reactions

Component	Volume/reaction
mRNA enrichment reaction (already in tube)	27 µl
RT Buffer, 5x*	8 µl
QIAseq FastSelect –Globin	1 µl
ERCC Control†	Optional
Nuclease-free water	1 µl
Total volume	**37 µl**

* From QIAseq Stranded Total RNA Lib Kit.

† ERCC Control RNA can be added according to the concentrations specified by the manufacturer. If added, replace the nuclease-free water (1 µl) with ERCC.

4. Incubate as described in Table 2, according to your input RNA quality and desired insert size.

Table 2. Combined QIAseq Stranded fragmentation and FastSelect hybridization protocol

Input RNA quality	Step	Insert size ~150–250 bp	Insert size ~350 bp
High quality (RIN >9)	1*	15 min at 95°C	3 min at 95°C
Moderate quality (RIN 5–6)	1*	10 min at 95°C	3 min at 95°C
FFPE or degraded sample (RIN <3)	1*	No fragmentation†	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	2 min at 75°C
	3	2 min at 70°C	2 min at 70°C
	4	2 min at 65°C	2 min at 65°C
	5	2 min at 60°C	2 min at 60°C
	6	2 min at 55°C	2 min at 55°C
	7	2 min at 37°C	2 min at 37°C
	8	2 min at 25°C	2 min at 25°C
	9	Hold at 4°C	Hold at 4°C

* Choose one option for the step 1 time, according to the input RNA quality and desired insert size.

† Also suitable for exosomal RNA or RNA of other origin with a size between 80–500 bp.

Important: Regardless of time and temperature chosen in step 1, steps 2–9 must be performed.

5. Refer to the *QIAseq Stranded mRNA Select Kit Handbook* and immediately proceed to "Protocol: First-strand Synthesis."
6. Follow the *QIAseq Stranded mRNA Select Kit Handbook* to perform all remaining library construction steps.

   Important: When removing globin, 2 additional cycles of CleanStart® 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.