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QIAseq® FastSelect™ –rRNA HMR and –Globin Handbook

Removal of rRNA and/or globin mRNA for RNA-seq applications from human/mouse/rat and other mammalian samples

Sample to Insight

QIAGEN

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Kit Contents

Human/Mouse/Rat (HMR)

QIAseq FastSelect –rRNA HMR Kit **Catalog no.** **Number of reactions**	**(24)** **334386** **24**	**(96)** **334387** **96**	**(384)** **334388** **384**
QIAseq FastSelect –rRNA HMR	3 x 8 µl	96 µl	4 x 96 µl

QIAseq FastSelect –Globin Kit **Catalog no.** **Number of reactions**	**(24)** **334376** **24**	**(96)** **334377** **96**	**(384)** **334378** **384**
QIAseq FastSelect –Globin	3 x 8 µl	96 µl	4 x 96 µl

QIAseq FastSelect –rRNA/Globin Kit **Catalog no.** **Number of reactions**	**(24)** **335376** **24**	**(96)** **335377** **96**	**(384)** **335378** **384**
QIAseq FastSelect –rRNA HMR	3 x 8 µl	96 µl	4 x 96 µl
QIAseq FastSelect –Globin	3 x 8 µl	96 µl	4 x 96 µl

Shipping and Storage

QIAseq FastSelect is shipped on blue ice or dry ice. Upon receipt, all components should immediately be stored in a constant-temperature freezer at –30 to –15°C. Under these conditions, the components are stable, without showing any reduction in performance and quality, until the date indicated on the box label.

Intended Use

All QIAseq FastSelect products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAseq FastSelect is tested against predetermined specifications to ensure consistent product quality.

Introduction

RNA-focused next-generation sequencing (NGS) enables a thorough investigation of both coding and noncoding RNAs. While performing stranded library preparation, significantly over-represented RNAs such as ribosomal RNA (rRNA) and globin messenger RNA (mRNA) must be avoided to facilitate optimal read allocation. QIAseq FastSelect is a breakthrough technology that rapidly and efficiently removes both cytoplasmic and mitochondrial rRNA and/or globin mRNA during NGS library preparation from 1 ng – 1 µg of human, mouse, rat, and other mammalian RNAs.

Cytoplasmic and mitochondrial rRNA comprise more than 80% of the total RNA in common biological samples, while globin mRNA is significantly overrepresented in whole-blood samples. As a result, commercial solutions exist to either enrich for poly(A)+ RNAs or deplete rRNA and/or globin. Existing strategies to remove rRNA and highly expressed mRNAs rely on probe-based depletion or enzymatic (RNase H) removal strategies. Unfortunately, these depletion methods are tedious, often taking more than 2 hours with extensive sample handling steps and are unable to work with formalin-fixed paraffin-embedded (FFPE) or fragmented samples.

Using the QIAseq FastSelect, the removal of rRNA and/or globin mRNA from human, mouse, rat, and other mammalian samples can be accomplished in just 14 minutes.

QIAseq FastSelect works with existing RNA-seq workflows for the removal of unwanted RNAs during the reverse transcription step of NGS library preparation. QIAseq FastSelect is

compatible with a broad range of commercially available stranded library preparation kits; in one step, QIAseq FastSelect removes up to 99% of unwanted cytoplasmic and mitochondrial rRNA and/or globin mRNA from human, mouse, rat, and other mammalian RNA libraries.

Principle and procedure

QIAseq FastSelect is designed for fast and selective removal of cytoplasmic and mitochondrial rRNA and/or globin mRNA from total RNA during NGS RNA library preparation. The simple one-step protocol calls for the FastSelect reagent to be directly combined with total RNA (1 ng – 1 µg) and any reaction buffers necessary for RNA heat fragmentation. After the optional heat fragmentation is performed, the reaction is gradually cooled to room temperature. The remaining library preparation-specific steps are then followed without the need for any additional enzymatic cleanups or RNA depletion steps. QIAseq FastSelect has been designed to work equally well with high-quality RNA or highly fragmented samples.

QIAseq FastSelect –rRNA HMR and –Globin species compatibility

In addition to human, mouse, and rat samples, QIAseq FastSelect –rRNA HMR has been tested with a variety of other RNA samples (Table 1). QIAseq FastSelect –Globin has been tested with whole blood total RNA samples from human, mouse, and rat, wherein 95–99% removal of globin mRNA is routinely observed. Similar to FastSelect –rRNA HMR, FastSelect –Globin may work on species beyond human, mouse, and rat. Importantly, when working with total RNA blood samples, QIAseq FastSelect –rRNA HMR must be used in combination with FastSelect –Globin. When working with mRNA-enriched samples, only FastSelect –Globin needs to be used.

Table 1. Summary of rRNA removal using QIAseq FastSelect –rRNA HMR

Species*	% rRNA reads (no FastSelect)	% rRNA reads (with FastSelect)	% knockdown
Human	91.8	1.6	98
Mouse	92.9	2.2	98
Rat	93.0	2.6	97
Chicken	88.3	16.3	82
Cow	70.0	1.2	98
Cynomolgus monkey (*Macaca fascicularis*)	90.4	10.6	88
Dog	87.8	9.1	90
Hamster	77.5	3.5	95
Horse	74.1	1.8	98
Pig	87.6	17.3	80
Rabbit	88.8	16.5	81
Sheep	87.3	4.0	95

* We do not recommend using QIAseq FastSelect –rRNA HMR for rRNA removal from roundworm (*Caenorhabditis elegans*) or zebrafish (*Danio rerio*). Laboratory tests suggest minimal rRNA removal from these species using FastSelect –rRNA HMR.

QIAseq FastSelect: Sample type and stranded library kit compatibility

QIAseq FastSelect has been designed to be compatible with total RNA- and mRNA-enriched samples isolated from cells, fresh/frozen tissue, FFPE tissue, whole blood, and serum/plasma samples, including exosomes. The QIAseq FastSelect reagent and protocol has been tested with a variety of commercially available stranded RNA library preparation kits from QIAGEN, Illumina®, NEB®, and Roche®. For questions regarding specific protocols for kits that are not detailed in the handbook, please contact QIAGEN technical support.

Protocol: FastSelect –rRNA HMR and/or –Globin with the QIAseq Stranded Total RNA Lib Kit

Important points before starting

- The QIAseq Stranded Total RNA Lib Kit (cat. no. 180743 or 180745) is required for use with this protocol.
- This protocol has been tested with 100 ng – 1 µg of total RNA.
- **Important**: When removing globin, 2 additional cycles of CleanStart® Library Amplification need to be performed.
- Refer to the *QIAseq Stranded RNA Library Kit Handbook* available at **www.qiagen.com/HB-2465**

Procedure

1. Thaw total RNA on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and return to ice.
2. Prepare the reagents required for the RNA fragmentation and QIAseq FastSelect rRNA and/or globin removal.
   2a. Thaw 5x RT Buffer, nuclease-free water from the QIAseq Stranded kit, and the tube(s) 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 2. 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 2. Setup of fragmentation/RNA Removal reactions

Component	Volume/reaction
Total RNA (100 ng – 1 µg)	Variable
RT Buffer, 5x*	8 µl
QIAseq FastSelect –rRNA HMR†	1 µl
QIAseq FastSelect –Globin†	1 µl
ERCC Control‡	Optional
Nuclease-free water	Bring total reaction volume to 37 µl
Total volume	**37 µl**

* From QIAseq Stranded Total RNA Lib Kit.

† Choose QIAseq FastSelect –rRNA HMR and/or QIAseq FastSelect –Globin.

‡ ERCC Control RNA can be added according to the concentrations specified by the manufacturer. If added, the total fragmentation/RNA Removal reaction volume should remain 37 µl.

4. Incubate as described in Table 3, according to input RNA quality and desired insert size.

Table 3. Combined QIAseq 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 time on step 1 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 RNA Library Kit Handbook* and immediately proceed to "Protocol: First-strand Synthesis".
6. Follow the *QIAseq Stranded RNA Library Kit Handbook* to perform all remaining library construction steps.

IMPORTANT 	When removing globin, 2 additional cycles of CleanStart Library Amplification need to be performed.

Protocol: FastSelect –Globin with the QIAseq Stranded mRNA Select Kit

Important points before starting

- The QIAseq Stranded mRNA Select Kit (cat. no. 180773 or 180775) is required for use with this protocol.
- **Important**: When removing globin, 2 additional cycles of CleanStart Library Amplification need to be performed.
- Refer to the *QIAseq Stranded mRNA Library Kit Handbook* available at **www.qiagen.com/HB-2464**

Procedure

1. From the *QIAseq Stranded mRNA Library 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 4. 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 4. 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 5, according to your input RNA quality and desired insert size.

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

IMPORTANT 	When removing globin, 2 additional cycles of CleanStart Library Amplification need to be performed.

Protocol: FastSelect –rRNA HMR and/or –Globin with TruSeq Stranded Library Preparation

Important points before starting

- The TruSeq® Stranded mRNA Library Prep (Illumina cat. no. 20020594 or 20020595) is required for use with this protocol.
  Note: With this protocol, do not perform mRNA purification. Instead, follow the steps outlined below before proceeding to "Synthesize First Strand cDNA" in the *TruSeq Stranded mRNA Reference Guide*. By doing this, a stranded, total RNA library preparation will be performed.
- This protocol has been tested with 100 ng – 1 µg of total RNA.
- **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.
- Refer to the *TruSeq Stranded mRNA Reference Guide* (1000000040498).

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect 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 QIAseq FastSelect –rRNA HMR
   - **Option 2 (remove globin)**: Add 1 µl of QIAseq FastSelect –Globin
   - **Option 3 (remove rRNA and globin)**: Add 1 µl of QIAseq FastSelect –rRNA HMR and 1 µl of QIAseq FastSelect –Globin

3. From the TruSeq Stranded mRNA Library Prep, add 14.5 µl FPF (when using option 1 or 2 in the previous step), or add 13.5 µl FPF (when using option 3 in the previous step), 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 from the sides of the tubes.
5. Incubate in a thermal cycler with a heated lid as described in Table 6.

   Important: Table 7 can be consulted to adjust RNA insert size. Irrespective of time at 94°C, steps 2–9 listed in Table 6 must be performed.

Table 6. Combined TruSeq Stranded fragmentation and FastSelect 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	2 min at 37°C
8	2 min at 25°C
9	Hold at 4°C

* The initial step at 94°C can be modified to permit longer RNA insert sizes. Refer to Table 7 for recommendations.
Note: The remaining steps 2–9 are performed regardless of the time at 94°C.

Table 7. Fragmentation time at 94°C for alternative RNA insert sizes

Time at 94°C*	Range of insert length (bp)	Median insert length (bp)	Average final library size (Bioanalyzer bp)
0 min	130–350	200	467
1 min	130–310	190	439
2 min	130–290	185	410
3 min	125–250	165	366
4 min	120–225	160	326
8 min	120–210	155	309
12 min	115–180	140	272

* The remaining steps 2–9 from Table 6 must be performed regardless of the time at 94°C.

6. 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 min)" is not applicable.
7. 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 reference guide.

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

Protocol: FastSelect –Globin with the TruSeq Stranded mRNA Library Prep

Important points before starting

- The TruSeq Stranded mRNA Library Prep (Illumina cat. no. 20020594 or 20020595) is required for use with this protocol.
- **Important**: It is highly recommended to dilute the Illumina adapters 2-fold compared to what is suggested in the Illumina protocol.
- **Important**: When removing globin, 2 additional cycles of library amplification need to be performed.
- 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 8.

Table 8. 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.

Protocol: FastSelect –rRNA HMR and/or –Globin with the NEBNext Ultra II Directional Library Prep Kit

Important points before starting

- The NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina (NEB cat. no. E7760S or E7760L) is required for use with this protocol.
- This protocol has been tested with 5 ng – 1 µg of total RNA.
- **Important**: When removing globin, 2 additional cycles of library amplification need to be performed.
- Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* (Version 3.1).

Procedure

1. Thaw the tube(s) 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 4 from the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual*, perform the following in place of steps 4.1.1 through 4.1.4:
   2a. Assemble the fragmentation and priming reaction described in Table 9 on ice in a nuclease-free tube.

Table 9. NEBNext Ultra II Stranded fragmentation and priming mix

Component	Volume/reaction
Total RNA (5 ng–1 µg)	4 µl
(lilac) NEBNext First Strand Synthesis Reaction Buffer*	4 µl
(lilac) Random Primers*	1 µl
Total volume	**9 µl**

* From NEBNext Ultra II Directional Library Prep Kit.

2b. To the assembled fragmentation and priming mix, add QIAseq FastSelect as follows:
- **Option 1 (remove rRNA)**: Add 1 µl of QIAseq FastSelect –rRNA HMR
- **Option 2 (remove globin)**: Add 1 µl of QIAseq FastSelect –Globin
- **Option 3 (remove rRNA and globin)**: Add 1 µl of QIAseq FastSelect –rRNA HMR and 1 µl of QIAseq FastSelect –Globin

2c. Mix thoroughly by pipetting up and down several times and then briefly centrifuge to collect residual liquid from the sides of the tubes

2d. Incubate in a thermal cycler with a heated lid as described in Table 10, according to your input RNA quality.

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

Table 10. Combined NEBNext Ultra II fragmentation and FastSelect hybridization protocol

Step	Intact RNA (RIN >7)	Partially degraded RNA (RIN 2–6)
1	15 min at 94°C	7–8 min at 94°C
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

3. Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* and immediately proceed to "First Strand cDNA Synthesis Reaction".
   Note: "First Strand cDNA Synthesis Reaction" is chapter 4.2 in Version 3.1 of the instruction manual.
4. 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.

Protocol: FastSelect –Globin with the NEBNext Poly(A) mRNA Magnetic Isolation Module and NEBNext Ultra II Directional Library Prep Kit

Important points before starting

- The NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB cat. no. E7490) is required for use with this protocol.
- The NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB cat. no. E7760S or E7760L) is required for use with this protocol.
- **Important**: When removing globin, 2 additional cycles of library amplification need to be performed.
- Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* (Version 3.1).

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 11.

Table 11. 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 3.1 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.

Protocol: FastSelect –rRNA HMR and/or –Globin with the KAPA RNA HyperPrep Kit

Important points before starting

- The KAPA® RNA HyperPrep Kit (Roche cat. no. KK8540 and KK8541) is required for use with this protocol.
- This protocol has been tested with 25 ng – 1 µg of total RNA.
- **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.
- Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet* (KR1350 – v2.17).

Procedure

1. Thaw the tube(s) from the QIAseq FastSelect kit. Mix by vortexing and then briefly centrifuge to collect residual liquid from the sides of the tubes.
2. From the KAPA RNA HyperPrep Kit, prepare the fragmentation and priming mix described in Table 12 at room temperature in a nuclease-free tube.

Table 12. KAPA RNA HyperPrep fragmentation and priming mix

Component	Volume/reaction
Total RNA (25 ng–1 µg)	9 µl*
Fragment, prime, and elute buffer (2X)†	10 µl
Total volume	**19 µl**

* Reduce volume to 8 µl if removing rRNA and globin.

† From KAPA RNA HyperPrep Kit.

3. To the assembled fragmentation and priming mix, add QIAseq FastSelect as follows:
   - **Option 1 (remove rRNA)**: Add 1 µl of QIAseq FastSelect –rRNA HMR
   - **Option 2 (remove globin)**: Add 1 µl of QIAseq FastSelect –Globin
   - **Option 3 (remove rRNA and globin)**: Add 1 µl of QIAseq FastSelect –rRNA HMR and 1 µl of QIAseq FastSelect –Globin
4. 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
5. Incubate in a thermal cycler with a heated lid as described in Table 13 according to your input RNA quality.

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

Table 13. Combined KAPA RNA HyperPrep fragmentation and FastSelect hybridization protocol

Input RNA type	Step	Time and temperature
Intact	1*	**Choose**: 8 min at 94°C *or* 6 min 94°C *or* 6 min at 85°C
Partially degraded	1†	1–6 min at 85°C
Degraded (e.g., FFPE)	1‡	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
	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

* Choose one option, depending if you want a desired mean library insert size of 100–200 bp (8 min at 94°C), 200–300 bp (6 min 94°C) or 300–400 bp (6 min at 85°C).

† For a desired mean library insert size of 100–300 bp.

‡ For a desired mean library insert size of 100–200 bp.

6. Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet* and immediately proceed to "1st Strand Synthesis", section 3 in v2.17.
7. Follow the *KAPA RNA 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.

Protocol: FastSelect –Globin with the KAPA mRNA HyperPrep Kit

Important points before starting

- The KAPA mRNA HyperPrep Kit (Roche cat. no. KK8580 and KK8581) is required for use with this protocol.
- **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.
- 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 14, according to your input RNA quality.

Table 14. 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.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
Adapter–dimer observed in final library QC		
a)	Depending on the library kit and RNA input amount, adapter–dimers may be observed.	Perform a second cleanup of the final library using the same beads-to-sample ratio as in the first cleanup.

Ordering Information

Product	Contents	Cat. no.
QIAseq FastSelect –rRNA HMR Kit (24)	For 24 reactions: cytoplasmic and mitochondrial rRNA removal reagent; supports human, mouse, and rat	334386
QIAseq FastSelect –rRNA HMR Kit (96)	For 96 reactions: cytoplasmic and mitochondrial rRNA removal reagent; supports human, mouse, and rat	334387
QIAseq FastSelect –rRNA HMR Kit (384)	For 384 reactions: cytoplasmic and mitochondrial rRNA removal reagent; supports human, mouse, and rat	334388
QIAseq FastSelect –Globin Kit (24)	For 24 reactions: globin mRNA removal reagent; supports human, mouse, and rat	334376
QIAseq FastSelect –Globin Kit (96)	For 96 reactions: globin mRNA removal reagent; supports human, mouse, and rat	334377
QIAseq FastSelect –Globin Kit (384)	For 384 reactions: globin mRNA removal reagent; supports human, mouse, and rat	334378
QIAseq FastSelect –rRNA/Globin Kit (24)	For 24 reactions: cytoplasmic and mitochondrial rRNA removal reagent and globin mRNA removal reagent; supports human, mouse, and rat	335376
QIAseq FastSelect –rRNA/Globin Kit (96)	For 96 reactions: cytoplasmic and mitochondrial rRNA removal reagent and globin mRNA removal reagent; supports human, mouse, and rat	335377

Product	Contents	Cat. no.
QIAseq FastSelect –rRNA/Globin Kit (384)	For 384 reactions: cytoplasmic and mitochondrial rRNA removal reagent and globin mRNA removal reagent; supports human, mouse, and rat	335378
QIAseq Stranded Total RNA Lib Kit (24)	For 24 stranded RNA-seq sequencing library preparation reactions: fragmentation, reverse transcription, second-strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment, and QIAseq Beads for library cleanups	180743
QIAseq Stranded Total RNA Lib Kit (96)	For 96 stranded RNA-seq sequencing library preparation reactions: fragmentation, reverse transcription, second-strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment, and QIAseq Beads for library cleanups	180745
QIAseq Stranded mRNA Select Kit (24)	For 24 stranded RNA-seq sequencing library preparation reactions: mRNA enrichment of total RNA, fragmentation, reverse transcription, second-strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment, and QIAseq Beads for library cleanups	180773

Product	Contents	Cat. no.
QIAseq Stranded mRNA Select Kit (96)	For 96 Stranded RNA-seq sequencing library preparation reactions: mRNA enrichment of total RNA, fragmentation, reverse transcription, second-strand synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment, and QIAseq Beads for library cleanups	180775

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
09/2019	Initial release
10/2019	Updated library amplification wording. Slightly modified shipping conditions.
02/2021	Corrected an error in the "Shipping and Storage" section. Editorial and layout changes. Updated the names of handbooks referenced (HB-2464 and HB-2465). Updated the version number of referenced Illumina Instruction Manual.

Notes

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