September 2018

QIAseq® FastSelect™ RNA Removal Kit

Inline removal of cytoplasmic and mitochondrial rRNA and/or globin mRNA during stranded RNA library preparation

Sample to Insight

QIAGEN

Contents

Handbook Revision History

Document Revision History	
R1 09/2018	Initial release

Kit Contents

Human

QIAseq FastSelect RNA Removal Kit Human Cyto & Mito rRNA	(24)	(96)	(384)
Catalog no. 333180	**THS-001Z-24**	**THS-001Z-96**	**THS-001Z-384**
Number of reactions	**24**	**96**	**384**
Human rRNA	30 µl	120 µl	4 x 120 µl

QIAseq FastSelect RNA Removal Kit Human Globin mRNA	(24)	(96)	(384)
Catalog no. 333180	**THS-002Z-24**	**THS-002Z-96**	**THS-002Z-384**
Number of reactions	**24**	**96**	**384**
Human Globin	30 µl	120 µl	4 x 120 µl

QIAseq FastSelect Multi-RNA Removal Kit Human Complete rRNA & Globin mRNA	(24)	(96)	(384)
Catalog no. 333280	**THS-201Z-24**	**THS-201Z-96**	**THS-201Z-384**
Number of reactions	**24**	**96**	**384**
Human rRNA	30 µl	120 µl	4 x 120 µl
Human Globin	30 µl	120 µl	4 x 120 µl

Mouse

QIAseq FastSelect RNA Removal Kit Mouse Cyto & Mito rRNA	(24)	(96)	(384)
Catalog no. 333180	**TMM-001Z-24**	**TMM-001Z-96**	**TMM-001Z-384**
Number of reactions	**24**	**96**	**384**
Mouse rRNA	30 µl	120 µl	4 x 120 µl

QIAseq FastSelect RNA Removal Kit Mouse Globin mRNA	(24)	(96)	(384)
Catalog no. 333180	**TMM-002Z-24**	**TMM-002Z-96**	**TMM-002Z-384**
Number of reactions	**24**	**96**	**384**
Mouse Globin	30 µl	120 µl	4 x 120 µl

QIAseq FastSelect Multi-RNA Removal Kit Mouse Complete rRNA & Globin mRNA	(24)	(96)	(384)
Catalog no. 333280	**TMM-201Z-24**	**TMM-201Z-96**	**TMM-201Z-384**
Number of reactions	**24**	**96**	**384**
Mouse rRNA	30 µl	120 µl	4 x 120 µl
Mouse Globin	30 µl	120 µl	4 x 120 µl

Rat

QIAseq FastSelect RNA Removal Kit Rat Cyto & Mito rRNA	(24)	(96)	(384)
Catalog no. 333180	**TRN-001Z-24**	**TRN-001Z-96**	**TRN-001Z-384**
Number of reactions	**24**	**96**	**384**
Rat rRNA	30 µl	120 µl	4 x 120 µl

QIAseq FastSelect RNA Removal Kit Rat Globin mRNA	(24)	(96)	(384)
Catalog no. 333180	**TRN-002Z-24**	**TRN-002Z-96**	**TRN-002Z-384**
Number of reactions	**24**	**96**	**384**
Rat Globin	30 µl	120 µl	4 x 120 µl

QIAseq FastSelect Multi-RNA Removal Kit Rat Complete rRNA & Globin mRNA	(24)	(96)	(384)
Catalog no. 333280	**TRN-201Z-24**	**TRN-201Z-96**	**TRN-201Z-384**
Number of reactions	**24**	**96**	**384**
Rat rRNA	30 µl	120 µl	4 x 120 µl
Rat Globin	30 µl	120 µl	4 x 120 µl

Storage

The QIAseq FastSelect RNA Removal Kit may have been shipped on dry ice or blue ice. Upon receipt, all components should be stored at –20°C in a constant-temperature freezer.

Intended Use

All QIAseq FastSelect RNA Removal Kit 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 RNA Removal Kit 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 overrepresented RNAs, such as ribosomal RNA (rRNA) and globin mRNA must be avoided to facilitate optimal read allocation. The QIAseq FastSelect RNA Removal Kit is a breakthrough technology that rapidly and efficient removes both cytoplasmic and mitochondrial RNA and/or globin mRNA during NGS RNA library preparation from 1 ng to 1 µg of total RNA.

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. When prepping libraries from both coding and noncoding RNAs, or from damaged samples, depletion methodologies are used. Unfortunately, these depletion methods are tedious sample pre-treatments, typically taking more than 2 hours with extensive handling.

QIAseq FastSelect RNA Removal Kit is an inline solution for the removal of unwanted RNAs during NGS library preparation using a broad range of commercially available stranded library prep kits. In one-step, QIAseq FastSelect removes virtually all unwanted cytoplasmic and mitochondrial rRNA and/or globin mRNA from RNA libraries, removing up to 99%, even in difficult samples such as formalin-fixed paraffin-embedded (FFPE). Simply hybridize the QIAseq FastSelect reagent during the NGS library preparation, and unwanted RNAs are eliminated from the library prep. QIAseq FastSelect is compatible with a broad range of commercially available stranded RNA library prep kits provided by QIAGEN, Illumina, New England Biolabs and KAPA Biosystems, and works across the entire RNA input range suggested by each kit (1 ng to 1 µg of total RNA, depending on the kit).

Principle and Procedure

QIAseq FastSelect is designed for fast, efficient removal of cytoplasmic and mitochondrial RNA and/or globin mRNA from total RNA during NGS RNA library preparation. Prior to RNA heat fragmentation, the FastSelect reagent is directly combined with total RNA (1 ng to 1 µg) and the library prep-specific buffers. Heat-fragmentation is then performed, and the reaction temperature is gradually cooled to room temperature. Following this, the remaining library prep-specific steps are followed. There is no need to perform any type of enrichment on the total RNA samples.

QIAseq FastSelect kit and sample-type compatibility

QIAseq FastSelect has been tested to be compatible with a variety of commercially available stranded RNA library prep kits (Table 1). In addition, QIAseq FastSelect has been verified to be compatible with a variety of total RNA samples isolated from cells, fresh/frozen tissue, FFPE tissue, whole blood and serum/plasma samples, including exosomes. Routinely, 95 to 99% depletion of cytoplasmic and mitochondrial rRNA and globin mRNA is observed.

Table 1. QIAseq FastSelect kit compatibility*

Vendor	Kit	Cat. No.	Total RNA range tested
QIAGEN	QIAseq Stranded Total RNA Lib Kit	180743, 180745	100 ng to 1 µg
Illumina	TruSeq Stranded	20020594, 20020595	100 ng to 1 µg
New England Biolabs	NEBNext® Ultra™ II Directional	E7760S, E7760L	5 ng to 1 µg
Kapa Biosystems	KAPA RNA HyperPrep	KK8540, KK8541	25 ng to 1 µg

*Generally speaking, QIAseq FastSelect is compatible with any stranded RNA library prep kit that begins with heat fragmentation of RNA. For questions regarding kits that are not listed, please contact QIAGEN technical support.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate SDSs available from the product supplier.

- **Stranded RNA library kit**:
  QIAseq Stranded Total RNA Lib Kit (QIAGEN, cat. no. 180743, 180745)
  TruSeq Stranded (Illumina, cat. no. 20020594, 20020595)
  NEBNext® Ultra™ II Directional (New England Biolabs, cat. no. E7760S, E7760L)
  KAPA RNA HyperPrep (Kapa Biosystems, cat. no. KK8540, KK8541)

Protocol: rRNA and/or Globin removal during QIAseq Stranded library preparation

Important points before starting

- The QIAseq Stranded Total RNA Lib Kit (QIAGEN, cat. no. 180743, 180745) is required for use with this protocol.
- This protocol has been tested with 100 ng to 1 µg of total RNA.
- Refer to the *QIAseq Stranded Total RNA Lib Kit Handbook*: **www.qiagen.com/HB-2465.**

Procedure

1. From the QIAseq Stranded Total RNA Lib Kit, dilute 1 µl of 1 M DTT to 0.4 M by adding 1.5 µl of RNase-free water. Discard this dilution after 48 hours.
2. 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.
3. To 100 ng – 1 µg of total RNA, 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.
4. Bring the volume of the reaction to 29 µl.
   Optional: Per the *QIAseq Stranded Total RNA Lib Kit Handbook*, ERCC Control RNA can be added according to the concentrations specified by the manufacturer. If added, the reaction volume should remain 29 µl.
5. From the QIAseq Stranded Total RNA Lib Kit, add 8 µl 5x RT Buffer to each sample. Briefly centrifuge, mix by pipetting up and down 10 times and centrifuge briefly again.
6. Incubate in a thermal cycler with a heated lid as described in Table 2, according to your input RNA quality and approximate insert size.

IMPORTANT: Regardless of time and temperature chosen in Step 1, Steps 2-9 must be performed.

Table 2. QIAseq Stranded fragmentation and 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	5 min at 37°C	5 min at 37°C
	8	5 min at 25°C	5 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.

7. Refer to the *QIAseq Stranded Library Kit Handbook* and immediately perform Step 5 from "RNA fragmentation and reverse transcription."
   Note: Step 5 is specifically "After fragmentation, add 1 µl RT Enzyme, 1 µl RNase Inhibitor and 1 µl diluted DTT (0.4 M). Briefly centrifuge, mix by pipetting up and down 10 times and centrifuge briefly again."
8. Follow the *QIAseq Stranded Library Kit Handbook* to perform all remaining library construction steps.
   Note: When removing Globin, 2 additional cycles of library amplification may need to be performed.

Protocol: rRNA and/or Globin removal during Truseq Stranded library preparation

Important points before starting

- TruSeq Stranded mRNA Library Prep (Illumina, cat. no. 20020594, 20020595) is required for use with this protocol.
  Note: 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*.
- This protocol has been tested with 100 ng to 1 µg of total RNA.
- Refer to the *TruSeq Stranded mRNA Reference Guide* (1000000040498) available at **support.illumina.com**.

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 from the sides of the tubes.

5. Incubate in a thermal cycler with a heated lid as described in Table 3.
   IMPORTANT: Table 4 can be consulted to adjust RNA insert size. Irrespective of time at 94°C, steps 2-9 listed in Table 3 must be performed.

Table 3. 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 for recommendations.
Note: The remaining steps 2-9 are performed regardless of the time at 94°C.

Table 4. 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 3 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 minutes)" is not applicable.
7. 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.

Protocol: rRNA and/or Globin removal during NEBNext Ultra II Directional library preparation

Important points before starting

- NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (New England Biolabs, cat. no. E7760S, E7760L) is required for use with this protocol.
- This protocol has been tested with 5 ng to 1 µg of total RNA.
- Refer to the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* available at **www.neb.com**.

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. From the NEBNext Ultra II Directional Library Prep Kit, assemble the fragmentation and priming reaction described in Table 5 on ice in a nuclease-free tube.

Table 5. NEBNext Ultra II Stranded fragmentation and priming mix

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

* From NEBNext Ultra II Directional Library Prep Kit.

3. To the assembled fragmentation and priming mix, 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.

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, according to your input RNA quality.
   IMPORTANT: Regardless of time and temperature chosen in Step 1, Steps 2-9 must be performed.

Table 6. NEBNext Ultra II fragmentation and 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	5 min at 37°C	5 min at 37°C
8	5 min at 25°C	5 min at 25°C
9	Hold at 4°C	Hold at 4°C

6. 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 Instruction Manual 1.0.
7. Follow the *NEBNext Ultra II Directional RNA Library Prep Kit for Illumina Instruction Manual* to perform all remaining library construction steps.
   Note: When removing Globin, 2 additional cycles of library amplification may need to be performed.

Protocol: rRNA and/or Globin removal during KAPA RNA HyperPrep library preparation

Important points before starting

- KAPA RNA HyperPrep Kit (Kapa Biosystems, cat. no. KK8540, KK8541) is required for use with this protocol.
- This protocol has been tested with 25 ng to 1 µg of total RNA.
- Refer to the *KAPA RNA HyperPrep Kit Technical Data Sheet* available at **www.kapabiosystems.com** or **sequencing.roche.com**.

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. From the KAPA RNA HyperPrep Kit, prepare the fragmentation and priming described in Table 7 at room-temperature in a nuclease-free tube.

Table 7. 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 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

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 8, according to your input RNA quality.
   IMPORTANT: Regardless of time and temperature chosen in Step 1, Steps 2-9 must be performed.

Table 8. KAPA RNA HyperPrep fragmentation and hybridization protocol

Input RNA type	Step	Insert size ~350 bp
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	5 min at 37°C
	8	5 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."
   Note: "1st Strand Synthesis" is Chapter 3 in v1.16 or v2.17.

7. Follow the *KAPA RNA HyperPrep Kit Technical Data Sheet* to perform all remaining library construction steps.
   Note: When removing Globin, 2 additional cycles of library amplification may 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: **http://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 kit and RNA input amount, adapter dimers may be observed.	Perform a second cleanup reaction of the final library.

Ordering Information

Product	Contents	Cat. no.
QIAseq FastSelect RNA Removal Kit Cyto & Mito rRNA Available for human, mouse or rat Available in 12, 96 or 384 reactions	Species-specific cytoplasmic and mitochondrial rRNA removal reagent	333180
QIAseq FastSelect RNA Removal Globin mRNA Available for human, mouse or rat Available in 12, 96 or 384 reactions	Species-specific globin mRNA removal reagent	333180
QIAseq FastSelect Multi-RNA Removal Kit Complete rRNA & Globin mRNA Available for human, mouse or rat Available in 12, 96 or 384 reactions	Species-specific cytoplasmic and mitochondrial rRNA removal reagent, Species-specific globin mRNA removal reagent	333280
QIAseq Stranded Total RNA Lib Kit (24)	For 24 Stranded RNA-seq sequencing library prep 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 prep reactions: fragmentation, reverse transcription, second-strand	180745

synthesis + end-repair + A-addition, adapter ligation, CleanStart PCR enrichment and QIAseq Beads for library cleanups

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.

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