December 2019

Supplementary Protocol

QIAseq® FastSelect™ –rRNA HMR and/or –Globin with the QIAseq UPX 3′ Transcriptome Kit

This protocol describes reverse transcription of purified RNA, with integrated rRNA and/or globin depletion, using the QIAseq UPX 3′ Transcriptome Kit (cat. no. 333088, 333089, or 333090) and the QIAseq FastSelect Kits for –rRNA HMR (cat. nos. 334386, 334387, 334388), –Globin (cat. nos. 334376, 334377, 334378), and –rRNA/Globin (cat. nos. 335376, 335377, 335378). While the QIAseq UPX 3′ Transcriptome Kit synthesizes cDNA via an anchored oligo-dT primer, residual amounts of rRNA may still be reverse transcribed. Additionally, when working with whole blood samples, cDNA will be synthesized from globin mRNA, as globin mRNAs are naturally polyadenylated.

IMPORTANT: Please consult the "Safety Information" and "Important Notes" sections in the *QIAseq UPX 3′ Transcriptome Handbook*, **www.qiagen.com/HB-2485**, and the *QIAseq FastSelect –rRNA HMR and –Globin Handbook*, **www.qiagen.com/HB-2670**, before beginning this procedure.

Equipment and reagents

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

- MagneSphere® Technology Magnetic Separation Stand (Promega, cat. no. Z5342)
- Nuclease-free pipette tips and tubes
- Microcentrifuge tubes (2 ml)
- Ice
- Microcentrifuge
- Thermal cycler

Important points before starting

- This protocol starts after "Protocol: Rebuffering of QIAseq Beads (RQ Beads)" in the *QIAseq UPX 3′ Transcriptome Handbook*.
- **Important**: Prior to use with the QIAseq UPX 3′ Transcriptome Kit, dilute QIAseq FastSelect –rRNA HMR and/or FastSelect –Globin to 0.08x using Nuclease-free Water.
- **Important**: Prepare beads prior to starting the reverse transcription or during the incubation steps of the protocol.
- This protocol can be used with low amounts of purified RNA (10 pg – 10 ng).
- When working with low amounts of purified RNA, 2 options are described:
  - CID-96S Plate: 96-well single-use Cell ID RT Plate
  - CID-384 Plate: 384-well single-use Cell ID RT Plate
- Ensure all reactions are mixed thoroughly and handled at the temperatures recommended in the protocol.
- Do not vortex reactions or reagents unless instructed.

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. Dilute an aliquot for each FastSelect tube (FastSelect –rRNA HMR and/or FastSelect –Globin) to 0.08x using 2 µl of FastSelect + 23 µl Nuclease-free Water. Mix by vortexing, and then briefly centrifuge to collect residual liquid from the sides of the tubes.
3. Prepare reagents required for the reverse transcription reactions. Thaw Cell Lysis Buffer, 3′ Trans RT Buffer and Nuclease-free Water at room temperature (15–25°C). Mix by flicking the tube. Centrifuge the tubes briefly to collect residual liquid from the sides of the tubes and keep at room temperature.

   EZ Reverse Transcriptase and RNase Inhibitor should be removed from the –30 to –15°C freezer and placed on ice just before preparation of the master mix. Both enzymes should be returned to the freezer immediately after use.

4. Prepare the reaction on ice as described in Table 1. Briefly centrifuge, mix by pipetting up and down 10 times, and then briefly centrifuge again.

Table 1. Preparation of RT premix for single-use Cell ID RT Plates (CID-96S Plate or CID-384 Plate)

Component	Each well*
Cell Lysis Buffer	1 µl
3′ Trans RT Buffer	1 µl
QIAseq FastSelect –rRNA HMR (0.08x)	0.5 µl
QIAseq FastSelect –Globin (0.08x)	0.5 µl
RNA	2 µl†
Total volume	**5 µl**

* A master mix can also be prepared. For this, prep 1.2x for each component, and add the RNA separately.
† Must be 2 µl or less. If less than 2 µl, make the difference up with Nuclease-free Water.

5. Incubate as described in Table 2 using a thermal cycler with a heated lid.

Table 2. QIAseq FastSelect hybridization protocol

Step	Time
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

6. Add 0.25 µl RNase Inhibitor and 0.25 µl EZ Reverse Transcriptase to each well.
7. Incubate as described in Table 3. Briefly centrifuge, mix by pipetting up and down 10 times, and then briefly centrifuge again.

Table 3. Reverse transcription incubation

Time	Temperature
10 min	25°C
90 min	42°C
15 min	70°C
∞	4°C

8. Upon completion of reverse transcription reactions, combine the synthesized cDNA from the different wells into one 2 ml microcentrifuge tube. Up to 96 wells can be combined in 1 tube.

   Note: The cDNA generated from each well of a Cell ID RT Plate contains a specific cell ID that enables tracking of that particular sample.

   Note: Minimally, the volume of the combined sample must be 100 µl. If the combined sample is not 100 µl, add Nuclease-free Water to bring the volume to 100 µl (indicated in Table 4 for 8 combined wells. As an example, when the cDNA from 8 wells is combined together, the total cDNA volume is 40 µl; due to this, 60 µl of Nuclease-free Water must be added).

Table 4. Addition of RQ Beads for cDNA cleanup

Number of wells combined	Nuclease-free Water	QIAseq Bead volume
8	60 µl	90 µl
24	0 µl	108 µl
96*	0 µl	432 µl

* When working with 384 wells, perform the cleanup as four sets of 96 wells. The supernatants will be combined after step 14, prior to the second cleanup.

9. Add 0.9x (volume) of RQ Beads to the combined cDNA synthesis reactions from step 8 (e.g., 90 µl beads to 100 µl synthesis reactions). Mix well by pipetting up and down 12 times.
10. Incubate for 10 min at room temperature.

    Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant.

    Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

    Important: Do not discard the beads, because they contain the DNA of interest.
11. Add 200 µl 80% ethanol. Rotate the tube 3 times to wash the beads. Carefully remove and discard the wash.
12. Repeat the ethanol wash.

    Important: Completely remove all traces of ethanol after this second wash. To do this, briefly centrifuge and then return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µl pipette tip, and then a 10 µl pipette tip to remove any residual ethanol.
13. With the tubes (caps opened) still on the magnetic stand, air-dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry.
14. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 25 µl Nuclease-free Water. Mix well by pipetting.
15. Return the tube/plate to the magnetic rack until the solution has cleared.

16. Transfer 23 µl of the supernatant to clean tubes.

    Important: When working with 384 wells, combine all 4 eluates to give 92 µl.

17. Adjust the supernatant volume to 100 µl using Nuclease-free Water.
18. Add 0.9x (volume) of RQ Beads. Mix well by pipetting up and down 12 times.
19. Incubate for 10 min at room temperature.
20. Place the tubes/plate on a magnetic rack for 5 min. After the solution has cleared, carefully remove and discard the supernatant.

    Note: Keep the beads on the magnetic stand while handling the supernatant and during the washing steps.

    Important: Do not discard the beads as they contain the DNA of interest.

21. Add 200 µl 80% ethanol. Rotate the tube 3 times to wash the beads. Carefully remove and discard the wash.
22. Repeat the ethanol wash.

    Important: Completely remove all traces of ethanol after this second wash. To do this, briefly centrifuge and then return the tubes or plate to the magnetic stand. Remove the ethanol first with a 200 µl pipette tip, and then a 10 µl pipette tip to remove any residual ethanol.

23. With the tubes (caps opened) still on the magnetic stand, air-dry at room temperature for 10 min.

    Note: Visually inspect that the pellet is completely dry. Ethanol carryover to the next step will affect reaction efficiency.

24. Remove the beads from the magnetic stand and elute the DNA from the beads by adding 13 µl Nuclease-free Water. Mix well by pipetting.
25. Return the tube/plate to the magnetic rack until the solution has cleared.
26. Transfer 11 µl of the supernatant to clean tubes.
27. From this point forward in the protocol, the procedures assume that all cDNA wells (either 8, 24, 96, or 384) have been combined into a single tube.
28. Proceed with "Optional Protocol: Quantitative Determination of Template Amplification" in the *QIAseq UPX 3′ Transcriptome Handbook*. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

    Note: If the number of cycles required for template amplification has already been determined, proceed with "Protocol: Template Amplification" in the *QIAseq UPX 3′ Transcriptome Handbook*. Alternatively, the samples can be stored at –30 to –15°C in a constant-temperature freezer.

Document revision history

Date	Changes
12/2019	Initial release

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.