Quick-Start Protocol September 2017

QIAseq™ Stranded Total RNA Library Kit

Part 1: Fragmentation, reverse transcription, second strand synthesis, end repair and A-addition

Immediately upon receipt, store the QIAseq Stranded Total RNA Kit (cat. nos. 180753 and 180755) at –30°C to –15°C. QIAseq Beads (cat. nos. 1107149 and 1107460) should be stored at 4°C (**do not freeze**). If stored under these conditions, kit and components are stable until the date indicated on the QC label.

Further information

- *QIAseq Stranded Total RNA Library Handbook*: **www.qiagen.com/HB-2465**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- For enrichment of polyadenylated mRNA prior to library preparation, follow the QIAseq Stranded mRNA Select Kit (cat. nos. 180773 and 180775) protocol.
- For stranded RNA-seq library prep without poly-A enrichment or when using previously rRNA-depleted samples, follow the protocol procedures the QIAseq Stranded Total RNA Kit (cat. nos. 180753 and180755) protocol.
- Use a thermal cycler with a heated lid to incubate reactions.
- To maximize output yields and minimize adapter dimer formation during bead handling, use 2 ml tubes.
- **Important**: Ensure reactions are thoroughly mixed by pipetting up and down, prepared and incubated at recommended temperatures.

- Ensure the QIAseq Beads are thoroughly mixed at all times. If a delay in the protocol occurs, simply vortex the beads.

RNA fragmentation and reverse transcription

1. Dilute 1 µl DTT (1M) DTT to 0.4M using 1.5 µl with RNase-free water. Discard after 48 h.
2. Dilute 1–100 ng of poly-A enriched mRNA or ribo-depleted RNA with RNase-free water to a total volume of 29 µl. Alternatively, use the complete reaction (29 µl) prepared from 100–5000 ng total RNA using the QIAseq Stranded mRNA Select Kit.

   Optional: Add ERCC Control RNA as outlined in the handbook.
3. Add 8 µl 5x RT Buffer to each sample and incubate as described in Tables 1.

Table 1. RNA fragmentation protocol

Step	Input RNA quality	Approximate insert size	
		150–250 bp	350 bp†
1*	High-quality (RIN >9)	15 min at 95°C	3 min at 95°C
1*	Moderate-quality (RIN 5–6)	10 min at 95°C	3 min at 95°C
1*	FFPE or degraded sample (RIN <3)	No fragmentation‡	No fragmentation‡
2	–	4°C	Hold

* Step 1 time (choose one option) depends on the quality of input RNA and the desired insert size.
† Adapted QIAseq bead purification ratios could be necessary (see handbook for details).
‡ Also suitable for exosomal RNA or RNA of other origin with a size of 80–500 bp.

4. After fragmentation, add 1 µl RT Enzyme, 1 µl RNase Inhibitor and 1 µl diluted DTT (0.4M). Incubate as described in Table 2.

Table 2. Reverse transcription protocol

Step	Temperature	Incubation time
1	25°C	10 min
2	42°C	15 min
3	70°C	15 min
4	4°C	Hold

5. After reverse transcription, add 56 µl of resuspended QIAseq Beads. Mix well by pipetting up and down 10 times.
6. Incubate for 5 min at room temperature.
7. Place the tubes on a magnetic rack. After the solution has cleared (~10 min or longer), carefully remove and discard the supernatant. **Important**: Do not discard the beads as they contain the DNA of interest.
8. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tube (2 to 3 times) to wash the beads. Carefully remove and discard the wash.
9. Repeat the ethanol wash. **Important**: Completely remove all traces of the ethanol wash after this second wash. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
10. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 5 to 10 min. **Note**: Visually inspect that the pellet is completely dry, but avoid over-drying.
11. Remove the tubes from the magnetic stand, and elute the DNA from the beads by adding 40 µl nuclease-free water. Mix well by pipetting.
12. Return the tubes to the magnetic rack until the solution has cleared.
13. Transfer 38.5 µl supernatant to clean tubes/plate. Alternatively, the samples can be stored at –20°C in a constant-temperature freezer overnight.

Second strand synthesis / end-repair / A-addition

1. Add 5 µl Second Strand Buffer and 6.5 µl Second Strand Enzyme Mix. Incubate as described in Table 3.

Table 3. Second strand synthesis / end-repair / A-addition protocol

Step	Temperature	Incubation time
1	25°C	30 min
2	65°C	15 min
3	4°C	Hold

2. After second strand synthesis, add 70 µl QIAseq Beads. Mix well by pipetting up and down 10 times.
3. Incubate for 5 min at room temperature.
4. Place the tubes on a magnetic rack. After the solution has cleared (~10 min or longer), carefully remove and discard the supernatant.

   Important: Do not discard the beads as they contain the DNA of interest.
5. With the tubes still on the magnetic stand, add 200 µl of 80% ethanol. Rotate the tubes (2 to 3 times) to wash the beads. Carefully remove and discard the wash.
6. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol wash after this second wash. Remove the ethanol with a 200 µl pipette first, and then use a 10 µl pipette to remove any residual ethanol.
7. With the tubes (caps opened) still on the magnetic stand, air dry at room temperature for 5 to 10 min.

   Note: Visually inspect that the pellet is completely dry, but avoid over-drying.
8. Remove the tubes from the magnetic stand, and elute the DNA from the beads by adding 52 µl nuclease-free water. Mix well by pipetting.
9. Return the tubes to the magnetic rack until the solution has cleared.
10. Transfer 50 µl supernatant to clean tubes/plate. Alternatively, the samples can be stored at –20°C in a constant-temperature freezer overnight. Proceed to strand-specific ligation in part 2 of the protocol.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.