Quick-Start Protocol September 2017

QIAseq™ Stranded mRNA Enrichment Kit

Immediately upon receipt, store the QIAseq Stranded Total RNA Kit (cat. nos. 180753 and 180755) at –30°C to –15°C. Buffers mRBB and Pure mRNA Beads provided with the QIAseq Stranded mRNA Enrichment Kit (cat. nos. 1105688 and 1105689) and QIAseq Beads (cat. nos. 1107149 and 1107460) should be stored at 4°C (**do not freeze**). Other buffers should be stored at room temperature (15–25°C). If stored under these conditions, kits and components are stable until the date indicated on the QC label.

Further information

- *QIAseq Stranded mRNA Enrichment Handbook*: **www.qiagen.com/HB-2464**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- With this protocol, mRNA can be isolated from 100–5000 ng total RNA.
- Vortex Pure mRNA Beads for 3 min (before first use) or 1 min (before subsequent uses) to ensure that the magnetic particles are fully resuspended.
- Heat a water bath or heating block to 70°C, and heat Buffer OEB to 70°C.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at room temperature (15–25 °C).
- For stranded RNA-seq library preparations without poly-A enrichment or when using previously rRNA-depleted samples, follow the protocols provided with the QIAseq Stranded Total RNA Kit (cat. nos. 180753 and 180755). Refer to the kit handbook at **www.qiagen.com** for additional protocols, required materials and kits.

To maximize output yields and minimize adapter dimer formation: (1) Use 2 ml tubes during bead handling if possible (2) Vortex the tubes instead of mixing the bead solutions with a pipette

mRNA enrichment using the QIAseq Stranded mRNA Enrichment Kit

1. Before starting, preheat Buffer OEB on a heating block/water bath at 70°C.
2. Vortex Pure mRNA Beads for 1 min to thoroughly resuspend. Vortex briefly again immediately before pipetting.
3. Dilute 100–5000 ng of total RNA into 250 µl of RNase-free water and add 1 µl RNase Inhibitor.
4. Add 250 µl Buffer mRBB and 25 µl of thoroughly resuspended Pure mRNA Beads and vortex for 5 s to mix. Immediately centrifuge for 5 s and incubate for 3 min at 70°C.
5. Remove the samples from 70°C and place at room temperature for 10 min.
6. Centrifuge briefly to collect the sample, and then transfer to a magnetic stand. Pellet the beads for 2 min on a magnetic stand and carefully discard the supernatant. Leave residual liquid in the tube to minimize bead loss.
7. Resuspend the beads with 400 µl Buffer OW2, mix by vortexing (low setting for 5 s) and centrifuge for 5 s to collect the sample. Pellet the beads for 2 min on a magnetic stand and carefully discard the supernatant. Leave residual liquid in the tube to minimize bead loss.
8. Repeat step 7 for a total of two washes with Buffer OW2.
9. Pipet 50 µl Buffer OEB to the sample and resuspend the beads by vortexing. Briefly centrifuge the tube to collect the liquid.
10. Incubate samples at 70°C for 3 min.
11. Remove the sample from 70°C and place at room temperature for 5 min.
12. Add 50 µl of Buffer mRBB to each sample and mix by vortexing.
13. Incubate at room temperature for 10 min.

14. Centrifuge the sample tube and pellet the beads on a magnetic stand. Wait until bead separation has been completed and remove the supernatant. Leave residual liquid in the tube to minimize bead loss.
15. Add 400 µl Buffer OW2 and resuspend the beads by vortexing.
16. Pellet the beads on a magnetic stand for 1 min and carefully discard the supernatant. Leave residual liquid in the tube to minimize bead loss.
17. Add 31 µl of Buffer OEB heated to 70°C to the sample pellet to elute the mRNA.
18. Resuspend the beads by vortexing.
19. Centrifuge the sample tube. Pellet the beads on a magnetic stand and transfer 29 µl of the supernatant to a new tube. The supernatant contains enriched, poly-adenylated RNA. This may be stored at –20°C or –70°C prior to library preparation.
20. Follow the *QIAseq Stranded Total RNA Quick-Start Protocols* (parts 1–3) to prepare stranded RNA-seq libraries with successfully enriched mRNA.

Scan QR code for handbook.

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