Quick-Start Protocol April 2022

RNeasy® Pure mRNA Bead Kit

Store columns and Buffers OW2 and OEB at room temperature (15–25°C) and Pure mRNA Beads and Buffer mRBB at 2–8°C.

Further information

- *RNeasy Pure mRNA Bead Handbook*: **www.qiagen.com/HB-1783**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for mRNA enrichment of total RNA.
- Vortex the bottle containing 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.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at 20–30°C. Steps 5–8 can be processed using a centrifuge **or** a magnetic rack.
- Symbols: ■ indicates processing by centrifugation using a small spin column; ▲ indicates processing using a microcentrifuge tube and a magnetic rack.

1. Determine the amount of starting RNA. Pipet total RNA into an RNase-free 2 ml microcentrifuge tube, and adjust the volume with RNase-free water (if necessary) to 250, 500, or 650 µl (see Table 1). **Optional**: Add 1 µl RNase inhibitor (4 U/µl) to the sample.
2. Add appropriate volumes of Buffer mRBB and Pure mRNA Beads (see Table 1), and vortex.

Table 1. Volumes of Buffer mRBB and Pure mRNA Beads required for mRNA binding

Amount of starting RNA (µg)	Volume of RNA solution (µl)	Volume of Buffer mRBB (µl)	Volume of Pure mRNA Beads (µl)
<5	250	250	25
≤50	250	250	50
≤100	250	250	75
≤250	500	500	150
≤500	500	500	200
≤1000	650	650	400

3. Incubate the sample for 3 min at 70°C in a water bath or heating block. This step disrupts the secondary structure of RNA.
4. Remove the sample from the water bath/heating block and place at room

temperature for 10 min. This step allows hybridization between the oligo-dT of the Pure mRNA Beads and the poly-A tail of the mRNA.

5. ■ Pellet the mRNA:bead complex by centrifugation for 2 min at maximum speed, and carefully remove the supernatant by pipetting. ▲ Briefly centrifuge the 2 ml sample tube to remove drops of liquid from the inside of the lid and place the tube on a magnetic rack, wait (approx. 2 min) until bead separation has been completed and remove the supernatant.
   Note: Save the supernatant until certain that satisfactory binding and elution of poly A^+ mRNA has occurred.
6. Resuspend the mRNA:bead pellet in 400 µl Buffer OW2 by vortexing or pipetting, and ■ pipet into a small spin column (provided). Centrifuge for 1 min at maximum speed. ▲ Pipet the solution into a 1.5 ml microcentrifuge tube. Place the tube on a magnetic rack, wait (approx. 1 min) until bead separation has been completed and remove the supernatant.
7. ■ Transfer the spin column to a new RNase-free 1.5 ml microcentrifuge tube (provided), and apply 400 µl Buffer OW2 to the column. Centrifuge for 1 min at maximum speed and discard the flow-through. ▲ Apply 400 µl Buffer OW2 to the pellet. Pipet the solution into a 1.5 ml microcentrifuge tube. Place the tube on a magnetic rack, wait (approx. 1 min) until bead separation has been completed and remove the supernatant.
8. ■ Transfer the spin column to a new RNase-free 1.5 ml microcentrifuge tube (provided). Pipet 20 µl hot (70°C) Buffer OEB into the column, pipet up and down 3–4 times to resuspend the beads and centrifuge for 1 min at maximum speed. ▲ Pipet 20 µl hot (70°C) Buffer OEB into a 1.5 ml microcentrifuge tube and pipet up and down 3–4 times to resuspend the beads. Place the tube on a magnetic rack, wait (approx. 1 min) until bead separation has been completed and remove the supernatant and collect it in a new 1.5 ml microcentrifuge.
   Note: The volume of Buffer OEB used depends on the expected or desired concentration of poly A^+ mRNA.
9. For higher yields, repeat step 8 using the eluate from step 8 (for higher RNA concentration) or using fresh Buffer OEB (for maximum yield).

Document Revision History

Date	Changes
03/2022	Inserted Table 1.