

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 (0.1–5 µg) into an RNase-free 2 ml microcentrifuge tube, and adjust the volume with RNase-free water (if necessary) to a volume of 250 µl. **Optional**: Add 1 µl RNase inhibitor (4U/µl) to the sample.
 2. Add 250 µl Buffer mRBB and 25 µl Pure mRNA Beads, and vortex.
 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
07/2021	Corrected the HB number in the trademarks line.

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