Quick-Start Protocol March 2016

GeneRead™ Pure mRNA 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

- *GeneRead Pure mRNA 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 QIAGEN® 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 (~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 (~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 (~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 (~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).

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