

RNeasy® Fibrous Tissue Midi Kit

RNeasy Fibrous Tissue Midi Kit (cat. no. 75742) is shipped at ambient temperature. The RNase-Free DNase Set box, containing RNase-free DNase, Buffer RDD, and RNase-free water, should be stored immediately upon receipt at 2–8°C. The remaining components of the RNeasy Fibrous Tissue Kit should be stored dry at room temperature (15–25°C). All components are stable for at least 9 months under these conditions.

For more information, additional and more detailed protocols, and safety information, please refer to the *RNeasy Fibrous Tissue Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- Unless otherwise indicated, perform the procedure, as well as all centrifugation steps, at room temperature (15–25°C). Work quickly.
 - Add 10 μ l β -mercaptoethanol (β -ME), or 20 μ l 2 M dithiothreitol (DTT), per 1 ml Buffer RLT before use. Buffer RLT containing DTT or β -ME can be stored at room temperature for up to 1 month.
 - Add 4 volumes of ethanol (96–100%) to Buffer RPE for a working solution.
 - Prepare DNase I stock solution. Dissolve the lyophilized DNase I in 550 μ l RNase-free water by injecting the RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex. Store DNase I as single-use aliquots at –20°C for up to 9 months or at 2–8°C for up to 6 weeks. Do not refreeze after thawing.
1. Heat water bath or heating block to 55°C.
 2. If using the TissueRuptor®, disrupt and homogenize \leq 250 mg tissue in 2 ml Buffer RLT. If using the TissueLyser instruments, disrupt and homogenize

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- ≤150 mg tissue in 1 ml Buffer RLT, and adjust volume to 2 ml with Buffer RLT.
3. Add 4 ml RNase-free water, then add 65 μ l of proteinase K. Mix, and incubate at 55°C for 20 min.
 4. Centrifuge at 3000–5000 x g for 5 min.
 5. Transfer supernatant to a new tube. Add 0.5 volumes of 96–100% ethanol, and mix. Do not centrifuge.
 6. Transfer 3 ml sample to RNeasy Midi column (in a 15 ml collection tube). Close lid, centrifuge for 5 min at 3000–5000 x g, and discard flow-through. Repeat step until complete lysate is used.
 7. Add 2 ml Buffer RW1 to RNeasy column. Close lid, centrifuge for 5 min at 3000–5000 x g, and discard flow-through.
 8. Mix 20 μ l DNase stock solution with 140 μ l Buffer RDD, add to RNeasy membrane, and incubate for 15 min at 20–30°C.
 9. Add 2 ml Buffer RW1 to RNeasy column. Close lid, centrifuge for 5 min at 3000–5000 x g, and discard flow-through.
 10. Add 2.5 ml Buffer RPE to RNeasy column. Close lid, centrifuge for 2 min at 3000–5000 x g, and discard flow-through.
 11. Add 2.5 ml Buffer RPE to RNeasy column. Close lid, centrifuge for 5 min at 3000–5000 x g.
 12. Place RNeasy column in a new 15 ml tube. Add 150 μ l RNase-free water, close lid, and centrifuge for 3 min at 3000–5000 x g.

Optional: Repeat elution with another volume of water or with RNA eluate.

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

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1067538 01/2011
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