Quick-Start Protocol March 2016

RNeasy® Fibrous Tissue Mini Kit

The RNeasy Fibrous Tissue Kit (cat. no. 74704) 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 if not otherwise stated on label.

Further information

- *RNeasy Fibrous Tissue Handbook*: **www.qiagen.com/HB-0485**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

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 µl β-mercaptoethanol (β-ME), or 20 µl 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 µl 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. Disrupt and homogenize ≤30 mg tissue in 300 µl Buffer RLT using the TissueRuptor®, TissueLyser LT or TissueLyser II.
3. Add 590 µl RNase-free water, then 10 µl proteinase K, mix and incubate at 55°C for 10 min.
4. Centrifuge at 10,000 x *g* for 3 min.
5. Transfer supernatant to new tube. Add 0.5 volumes of 96–100% ethanol, and mix. Do not centrifuge.
6. Transfer 700 µl of sample to RNeasy Mini column (in a 2 ml collection tube). Close lid, centrifuge for 15 s at ≥8000 x *g* and discard flow-through. Repeat step until complete lysate is used.
7. Add 350 µl Buffer RW1 to RNeasy column. Close lid, centrifuge for 15 s at ≥8000 x *g* and discard flow-through.
8. Mix 10 µl DNase stock solution with 70 µl Buffer RDD, add to RNeasy membrane and incubate for 15 min at 20–30°C.
9. Add 350 µl Buffer RW1 to RNeasy column. Close lid, centrifuge for 15 s at ≥8000 x *g* and discard flow-through.
10. Add 500 µl Buffer RPE to RNeasy column. Close lid, centrifuge for 15 s at ≥8000 x *g* and discard flow-through.
11. Add 500 µl Buffer RPE to RNeasy column. Close lid, centrifuge for 2 min at ≥8000 x *g*.

    Optional: Place RNeasy column in new 2 ml tube, close lid and centrifuge at full speed for 1 min.
12. Place RNeasy column in new 1.5 ml tube. Add 30–50 µl RNase-free water, close lid and centrifuge for 1 min at ≥8000 x *g*.

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

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