Quick-Start Protocol March 2017

RNeasy® PowerLyzer® Tissue&Cells Kit (50)

All reagents and components of the RNeasy PowerLyzer Tissue&Cells Kit should be stored at room temperature (15–25°C).

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Warm Solution TR1 to 37°C for 5–10 minutes to dissolve any precipitate.
- Prepare Solution TR1 by adding 10 µl β-mercaptoethanol (βME) for every 1 ml of Solution TR1 for each sample to be processed. Solution TR1/ βME should be prepared fresh each time according to the number of samples being processed.
- Perform all steps at room temperature (15–30°C). Use a standard microcentrifuge. If using a refrigerated centrifuge, do not allow the centrifuge to cool.
- Homogenized samples in Solution TR1 may be stored at –65° to –90°C for up to 2 months until ready to use.

1. Properly identify each Ceramic Bead Tube on both the cap and on the side.
2. Homogenize tissue samples following ONLY one of the methods (a–d) described below. For alternative homogenization methods, please contact QIAGEN Technical Services.

   a) PowerLyzer 24 or other bead beater

   1) In a PowerLyzer Ceramic Bead Tube, 2.8 mm (provided), add 300 or 600 µl of Solution TR1/βME, according to Table 2 in the Handbook. Chill the tubes on ice or in a cooling block.
   2) Weigh and add tissues to the Bead Tube. Keep chilled until ready to homogenize.
   3) Place Bead Tubes into the Tube Holder of the PowerLyzer 24. The tubes must be balanced in the Tube Holder. Homogenize the tissue for 2 cycles at 3500 rpm for 45 s each with 30 s dwells between cycles.
   4) Centrifuge the Bead Tubes containing the tissue lysate at 13,000 x *g* for 1 min. Transfer the lysate to a new 2 ml collection tube (provided)

   b) Rotor-stator or Polytron homogenizer

   1) Weigh the tissues and place into a vessel aptly sized for your homogenizer.

2) Add 300 or 600 µl of Solution TR1/βME, according to Table 2 in the Handbook.
3) Homogenize for 30–40 s until the tissue is completely liquefied and no visible particulates remain.
4) Transfer the lysate to a new 2 ml collection tube (provided).

c) Liquid nitrogen and mortar and pestle

1) Weigh the tissues and place into the pre-chilled mortar.
2) Add liquid nitrogen and homogenize the tissue to a fine powder.
3) Resuspend powdered tissue with 300 or 600 µl of Solution TR1/βME, according to Table 2 in the Handbook. Transfer to a 2 ml collection tube (provided).
4) Shear genomic DNA using a 20-gauge needle on a 1 cc syringe by moving the lysate in and out of the syringe at least 10 times or until the sample loses viscosity.

d) Homogenization of cells

1) Collect cells from culture medium and perform a cell count to determine the correct volume of Solution TR1 to use.
2) Pellet cells at 2000 x *g* for 5 min. Wash cells once with phosphate buffered saline to remove the culture medium. Pellet the cells again at 2000 x *g* for 5 min.
3) Add 300 or 600 µl of Solution TR1/βME, according to Table 2 in the Handbook, and transfer sample to a 2 ml collection tube (provided).
4) Vortex for 2 min to resuspend cells. No visible cell debris should remain.

3. Add 1 equal volume (300 or 600 µl) of Solution TR2 to the lysate. Mix by pipetting.
4. Transfer 600 µl of lysate to an MB RNA Spin Column. Centrifuge at ≥10,000 x *g* for 1 min. Discard flow through and place the MB RNA Spin Column back into the 2 ml collection tube. If you used 600 µl each of Solutions TR1 and TR2, repeat this step.
5. Wash the MB RNA Spin Column with 500 µl of Solution WB. Centrifuge for 1 min at ≥ 10,000 x *g*. Transfer the Spin Filter to a new 2 ml collection tube (provided).
6. Wash the MB RNA Spin Column with 500 µl of Solution RW. Centrifuge for 1 min at ≥ 10,000 x *g*. Discard flow through. Place Spin Filter back into the 2 ml collection Tube.
7. Repeat step 6.
8. Centrifuge the MB RNA Spin Column for 2 min at 13,000 x *g* to dry the membrane. Transfer the MB RNA Spin Column to a new 2 ml collection tube (provided).
9. Add 50–100 µl of Solution RNase-free water directly onto the Spin Column membrane. Incubate for 1 min at room temperature. Centrifuge for 1 min at ≥ 10,000 x *g*. The RNA is now ready for downstream applications and can be stored at –65° to –90°C.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, RNeasy®, PowerLyzer® (QIAGEN Group). 1104504 03/2017 HB-2226-002 © 2017 QIAGEN, all rights reserved.