November 2022

Quick-Start Protocol

EZ1&2™ RNA Tissue Mini Kit

For use with EZ2® Connect instruments

For usage of EZ1&2 RNA Tissue Mini Kit with EZ1® instruments, please refer to the handbook (**www.qiagen.com/HB-0115**) and quick-start protocol (**www.qiagen.com/HB-0785**).

The EZ1&2 RNA Tissue Mini Kit (cat. no. 959034) containing RNase-free DNase I and RNase-free water should be stored immediately upon receipt at 2–8°C. The remaining kit components should be stored dry at room temperature (15–25°C).

Further information

- *EZ1&2 RNA Tissue Mini Kit for use with EZ2 Connect instruments*: **www.qiagen.com/HB-2976**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- If purifying RNA from cell lines rich in RNases or from tissue, we recommend adding either β-mercaptoethanol (β-ME) or 2 M dithiothreitol (DTT) to Buffer RLT before use (10 µL β-ME or 20 µL DTT per 1 mL Buffer RLT). 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 to obtain a working solution.
- Before loading reagent cartridges into the EZ2 instrument, invert the cartridges 4 times to mix the magnetic particles, and then tap to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

Procedure

For processing tissue

1. Add 300 µL Buffer RLT to tissue sample.
2. Disrupt and homogenize with appropriate method as described in Table 1. Proceed with step 3.

Table 1. Amount of starting material and disruption and homogenization method

Sample	Amount of starting material	Disruption and homogenization
Cells		
Cultured animal or human cells	10 – 1 x 10^6 cells	Vortex (≤1x10^5 cells); QIAshredder, TissueRuptor®, TissueLyser LT, TissueLyser II, or needle and syringe (>1x10^5 cells)
Human white blood cells	10 – 2 x 10^6 cells	
Tissue, flash frozen*		
Easy-to-lyse	≤10 mg	TissueLyser LT, TissueLyser II, TissueRuptor, or mortar and pestle followed by QIAshredder, or needle and syringe
High-cell density (e.g., spleen)	≤5 mg	
Tissue, RNAprotect® or Allprotect® stabilized†		
Easy-to-lyse	≤4–6 mg	
High cell density (e.g., spleen)	≤2–3 mg	

* Using fresh tissue is not recommended unless it is homogenized in Buffer RLT immediately, since RNA in unstabilized fresh tissue is not protected from degradation.

† Since RNAprotect or Allprotect stabilized tissues are partially dehydrated, a lower amount is used as starting material.

For processing cells

1. Harvest cells as a cell pellet or, for cells grown in a monolayer, aspirate the cell-culture medium from the cell-culture vessel (up to 10 cm in diameter). Add 300 µL Buffer RLT to either the pellet or the cell-culture vessel, vortex or pipet to mix, and homogenize.
2. Centrifuge the lysate for 3 min at maximum speed. Carefully remove the supernatant by pipetting and transfer to a 2 mL sample tube (supplied). Proceed with step 3.

Automated purification on the EZ2 Connect

3. Turn on the EZ2 Connect instrument.
4. Tap **RNA** on the Applications panel, select **EZ1&2 RNA Tissue Mini Kit**, and press **Next**.
5. Follow onscreen instructions for selection of protocol, parameter definition, sample position selection, sample IDs, and worktable setup.
6. Load the EZ1&2 RNA Tissue Mini Kit reagent cartridges into the EZ2 Connect Cartridge Rack.
7. Open instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
8. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack):
   - Position A: Opened 2.0 mL tube with sample
   - Position B: Tip holder with inserted tip
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube
9. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.

   The display will show "Protocol finished" when the run is completed. Select **Finish**. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used EZ2 cartridge including the liquid waste.

   Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
10. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

Document Revision History

Date	Changes
November 2022	Initial release

Scan QR code for handbook.

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