Quick-Start Protocol December 2021

EZ2® RNA/miRNA Tissue/Cells Kit

For use with EZ2 Connect instruments

The EZ2 RNA/miRNA Tissue/Cells Kit (cat. no. 959035) is shipped at room temperature (15–25°C). Upon receipt, store the DNase I at 2–8°C. Store all other kit components dry at room temperature.

Further information

- *EZ2 RNA/miRNA Tissue/Cells Kit Handbook*: **www.qiagen.com/HB-2972**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **www.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.
- When using RNase-Free DNase I for the first time, reconstitute them as described in the handbook. Mixing should only be carried out by gently inverting the vial.
- Before adding DNase into the reagent cartridges and loading them into the EZ2 Connect instrument, invert the cartridges 4 times to mix the magnetic particles and then tap to deposit the reagents at the bottom of the wells. Make sure that the magnetic particles are completely resuspended.
- In the following procedure, text marked with ● denotes RNA purification from cells, and text marked with ▲ denotes RNA purification from tissue samples.

Procedure

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 diameter). Add 300 µl Buffer RLT to either the pellet or the cell-culture vessel, and homogenize (Table 1).
2. ▲ Add 300 µl Buffer RLT to tissue sample, and then disrupt and homogenize (Table 1).
3. Add 75 µl RNase-free Water and 25 µl Proteinase K, and mix and incubate for 10 min at room temperature. In the meantime, prepare the worktable as described below.

   Note: See Table 1 for the amount of starting material, and disruption and homogenization methods. Using more than the maximum recommended amount may result in reduced RNA yields and purity.

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

Sample	Amount of starting material	Buffer RLT	RNase-free water	Proteinase K	Disruption/homogenization
Pelleted Cells					
Cultured animal or human cells Human white blood cells	≤5 x 10^6	300 µl	75 µl	25 µl	Vortex (≤1x10^5 cells); QIAshredder, TissueRuptor® II, TissueLyser LT, TissueLyser II, or needle and syringe (>1x10^5 cells)
Tissue, flash frozen*					
Easy-to-lyse High-cell density (e.g., spleen)	≤30 mg ≤10 mg	300 µl	75 µl	25 µl	TissueLyser LT, TissueLyser II, TissueRuptor II, or mortar and pestle followed by QIAshredder or needle and syringe
Tissue, RNAprotect® stabilization reagent or Allprotect® stabilized†					
Easy-to-lyse High-cell density (e.g., spleen)	≤15 mg ≤5 mg	300 µl	75 µl	25 µl	

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

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

EZ2 Connect preparation

4. Turn on the EZ2 Connect instrument.
5. Tap on the RNA Applications panel and select the EZ2 RNA/miRNA Tissue/Cells Kit and press **Next**. Follow the on-screen instructions for selection of protocol, parameter definition, sample position selection, sample IDs, and worktable setup.
6. Add 20 µl DNase to well 5 of the RNA/miRNA Tissue/Cells reagent cartridges and load it into the EZ2 Connect Cartridge Rack (Position labels are engraved on the EZ2 Connect Cartridge Rack).
7. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
8. Remove the caps of all tubes and prepare the EZ2 Connect Tip Rack as follows (positions are labelled by engravings on the EZ2 Connect Tip Rack):
9. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the protocol following instructions on the instrument display.
   - Position A: 2 ml sample tube containing the sample from step 3
   - Position C: Tip holder with Filter Tip
   - Position D: 1.5 ml tube
10. The display will show "Protocol finished" when the run is completed. Select **Finish**.

    Open the instrument hood. Remove the 1.5 ml tube containing 50 or 100 µl purified RNA from position D 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.
11. Perform regular maintenance after each run. Press **Finish** to return to the Home Screen.

Document Revision History

Date	Changes
12/2021	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.