Quick-Start Protocol March 2016

EZ1® RNA Cell and EZ1 RNA Tissue Mini Kits

The box of the EZ1 RNA Cell Mini Kit and EZ1 RNA Tissue Mini Kit (cat. nos. 958034 and 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 RNA Handbook*: **www.qiagen.com/HB-0115**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The EZ1 RNA Cell Mini Kit and EZ1 RNA Tissue Mini Kit require use of the EZ1 Advanced XL RNA Card with the EZ1 Advanced XL, or use of the EZ1 Advanced RNA Card with the EZ1 Advanced, or use of the EZ1 RNA Card with the BioRobot® EZ1.
- 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.
- EZ1 instruments should only be switched on after an EZ1 Card is inserted. Make sure that the EZ1 Card is completely inserted otherwise essential instrument data could be lost, leading to a memory error. EZ1 Cards should not be exchanged while the instrument is switched on.
- Symbols: RNA purification from ● cells/▲ tissue samples.

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, vortex or pipet to mix and homogenize. ▲ Add 300 µl Buffer RLT to tissue sample, then disrupt and homogenize.

   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.

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).

3. Carry out RNA purification using an EZ1 instrument with the appropriate EZ1 Card, according to the *EZ1 RNA Handbook*.

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, RNA*later*® 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 RNA*later* or Allprotect stabilized tissues are partially dehydrated, a lower amount is used as starting material.

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