Quick-Start Protocol April 2016

AllPrep® DNA/RNA/miRNA Universal Kit, Part 1

The AllPrep DNA/RNA/miRNA Universal Kit (cat. no. 80224) should be stored dry at room temperature (15–25°C) and is stable for at least 9 months under these conditions if not otherwise stated on label. DNase I should be stored at 4–8°C upon arrival.

Further information

- *AllPrep DNA/RNA/miRNA Universal Handbook*: **www.qiagen.com/HB-1295**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- If purifying RNA from tissue or RNase-rich cell lines, add either 10 µl β-mercaptoethanol (β-ME) or 20 µl 2M dithiothreitol (DTT) to 1 ml Buffer RLT Plus, before use. Buffer RLT Plus containing β-ME or DTT can be stored at room temperature for up to 1 month.
- For preparation of buffers, see the *AllPrep DNA/RNA/miRNA Universal Handbook*.
- Foaming can be reduced by adding Reagent DX (cat. no. 19088) at a final concentration of 0.5% (v/v) before disruption and homogenization.

Sample disruption and homogenization of cells or tissue

1. **Starting material**: Do not use more than 30 mg tissue* or 1 x 10^7 cells (in the case of cultured cells), or 1.5 ml of whole blood (a maximum of 1 x 10^7 leukocytes can be processed). If using whole blood, cellular components must first be separated, for example by erythrocyte lysis or ficoll gradient. Disrupt the tissue or cells and homogenize the lysate in the appropriate volume of Buffer RLT Plus (see Table 1). For details on disruption and homogenization, see the *AllPrep DNA/RNA/miRNA Universal Handbook*.

* Do not use more than 20 mg of tissue stabilized in RNA*later*® or Allprotect Tissue Reagent.

2. Transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 ml collection tube (supplied). Close the lid gently and centrifuge for 30 s at full speed (maximum 20,000 x *g*).
3. Use the flow-through for RNA purification. (See "Total RNA purification" in the *Quick-Start Protocol AllPrep DNA/RNA/miRNA Universal Kit, Part 2*.)
4. Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied). Store at room temperature (15–25°C), or at 4°C if not performing DNA purification until later. For details on DNA purification, see "Genomic DNA purification" in the *Quick-Start Protocol AllPrep DNA/RNA/miRNA Universal Kit, Part 2*.

   Note: Do not store the column at room temperature (15–25°C) or 4°C for long periods. Do not freeze the column.

Table 1. Volumes of Buffer RLT Plus for sample disruption and homogenization

Sample	Amount	Dish	Buffer RLT Plus*	Disruption† and homogenization
Animal cells	<5 x 10^6	<6 cm	350 µl	Add Buffer RLT Plus, vortex (≤1 x 10^5 cells); or use the QIAshredder, TissueRuptor®, TissueLyser LT, TissueLyser II or a needle and syringe
	≤1 x 10^7	6–10 cm	600 µl	
Animal tissues*	<10 mg	–	350 µl	Add Buffer RLT Plus, use the TissueLyser LT, TissueLyser II, TissueRuptor or a mortar and pestle, followed by the QIAshredder or a needle and syringe
	≤30 mg	–	600 µl	
Whole blood	Up to 0.5 ml (up to 2 x 10^6)	–	350 µl	Add Buffer RLT Plus, use the QIAshredder, TissueRuptor, TissueLyser LT, TissueLyser II or a needle and syringe
	0.5–1.5 ml (2 x 10^6–1 x 10^7)	–	600 µl	

* Use 600 µl Buffer RLT Plus for tissues stabilized in RNA*later* or Allprotect Tissue Reagent.

† For optimal yields, thorough homogenization is required (e.g., using the TissueRuptor, TissueLyser LT or TissueLyser II).

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