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Qiagen All-Prep DNA/RNA Mini Kit V.1

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The AllPrep DNA/RNA Mini Kit is designed to purify genomic DNA and total RNA simultaneously from a single biological sample. Lysate is first passed through an AllPrep DNA spin column to selectively isolate DNA and then through an RNeasy spin column to selectively isolate RNA. Pure DNA and RNA are purified from the entire sample, in contrast to other procedures where either the biological sample or the purified total nucleic acids is divided into two before being processed separately. The kit is compatible with small amounts of a wide range of animal cells and tissues.

The AllPrep DNA/RNA Mini Kit allows the parallel processing of multiple samples in less than 40 minutes. Time-consuming and tedious methods, such as CsCl step-gradient ultracentrifugation and alcohol precipitation steps, or methods involving the use of toxic substances, such as phenol and/or chloroform, are replaced by the AllPrep DNA/RNA procedure.

Genomic DNA purified with the AllPrep DNA/RNA procedure has an average length of 15–30 kb depending on homogenization conditions. DNA of this length is particularly suitable for PCR, where complete denaturation of the template is important to achieve the highest amplification efficiency. The purified DNA is ready to use in any downstream application, including:

- Next-generation sequencing (NGS)
- PCR
- Southern, dot and slot blot analyses
- Comparative genome hybridization (CGH)
- Genotyping, SNP analysis

HB-2697-003_HB_AllPrep_DNARNA_Mini_1120_WW.pdf

George Testo 2022. Qiagen All-Prep DNA/RNA Mini Kit. **protocols.io** https://protocols.io/view/qiagen-all-prep-dna-rna-mini-kit-cafqsbmw

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Protocols

- Extraction List
- Reagent Calculations
- Buffer RLT Plus
- Buffer RPE
- Buffer AW1
- Buffer AW2

Reagents

- 1 x conical of **prepared** Buffer RLT Plus
- 1 x conical of **prepared** Buffer RPE
- 1 x conical of **prepared** Buffer AW1
- 1 x conical of **prepared** Buffer AW2

Supplies

- Lysis matrix E tubes
- DNA + RNA spin columns
- 1.5mL + 2.5mL collection tubes
- 1 x biohazard trash bags
- 1 x biohazard trash holders
- 1 x black sharpie

Equipment

- 1000uL (traditional) pipettes, tip boxes, & tips
- 200uL (traditional) pipettes, tip boxes, & tips
- 20uL (traditional) pipettes, tip boxes, & tips
- Conical fliprack

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.

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Homogenization 3m 30s

1 

Add **600 µL** of Buffer RLT Plus **Qiagen Catalog #1053393** to Lysing Matrix E tubes.

2 Place the entire tissue or a portion into Lysing Matrix E tubes for homogenization.

2.1 **For Swab(s):** pick up with tweezers and cut with surgical scissors sterilized in a Germinator 500. Place the cut end of a single swab into the Lysing Matrix E tube.

2.2 **For Nasal Lavage(s):** Thoroughly thaw the lavage and pipette up and down 6 times. Then transfer **500 µL** of the lavage to the Lysing Matrix E tube.

3 

Homogenize the lysate in Buffer RLT Plus (do not use more than 30 mg of tissue).

3.1 Use a homogenizer designed for Lysing Matrix E tubes.

3.2 Use a disruption setting that works best for your tissue (i.e. fecal, lung, etc).

4 3m

Centrifuge the lysate for **00:03:00** at maximum speed. Carefully remove supernatant by pipetting and transfer it to the All-Prep DNA spin column placed in a 2mL collection tube.

4.1 Close the lid gently, and centrifuge for **00:00:30** at **10.000 rpm** . 30s

Note: Make sure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane.

5 Place the All-Prep DNA spin column in a new 2mL collection tube, and store at room temperature (15-25C) or a **4 °C** for later DNA purification in the following steps.

Note: Do not store the All-Prep DNA spin column at room temperature or at 4C for long periods of time. Do not freeze the column.

Total RNA Purification 4m 45s

6 Add **1 volume (~1x) of 70% Ethanol** to the flowthrough from step 5 and mix well by pipetting. Do not centrifuge. Proceed immediately to step 7.

If some lysate was lost during homogenization, adjust the volume of ethanol.

Note: Precipitates may be visible after addition of ethanol, but this does not affect the procedure. For maximum RNA yields from live, use 50% ethanol instead of 70%.

7 Transfer up to **700 µL** of the sample, including any precipitate that may have formed, to a RNeasy spin column placed in a 2mL collection tube. Close the lid gently, and centrifuge for 15s **00:00:15** at **10.000 rpm** . Discard the flowthrough.

7.1 Reuse the collection tube in step 8.

Note: If the sample volume exceeds 700uL, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

8 15s

Buffer

Add **700 µL** of RW1 **Qiagen Catalog #1053394** to the RNeasy spin column.

Close the lid gently, and centrifuge for **00:00:15** at **10.000 rpm** to wash the spin column membrane. Discard the flow-through.

9 15s

Buffer

Add **500 µL** of RPE **Qiagen Catalog #1018013** to the RNeasy spin column.

Close the lid gently, and centrifuge for **00:00:15** at **10.000 rpm** to wash the spin column membrane. Discard the flow-through.

9.1 Reuse the collection tube in step 7.

Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use.

10 2m

Buffer

Add **500 µL** of RPE **Qiagen Catalog #1018013** to the RNeasy spin column.

Close the lid gently, and centrifuge for **00:02:00** at **10.000 rpm** to wash the spin column membrane.

Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions or usage of resultant RNAs and or DNAs.

Note: After centrifugation, carefully remove the RNeasy spin column from the collection

tube so that the column does not contact the flow-through; carryover ethanol may occur.

11 Place the RNeasy spin column in a new 2mL collection tube and discard the old collection tube with the flowthrough. Centrifuge at full speed for **00:01:00** . 1m

Note: Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 10.

12 Place the RNeasy spin column in a new 1.5ml collection tube. Add **30-50 µL** of RNAse-free Water **Contributed by users** directly to the spin column membrane. Close the lid gently, and centrifuge for **00:01:00** at **10.000 rpm** to elute the RNA. 1m

12.1

If expected RNA yield is >30ug, repeat step 12 using another **30-50 µL** of RNAse-free Water **Contributed by users** , or using the eluate from step 12 (if high RNA concentration is required).

Genomic DNA Purification 2m 15s

13 15s

Buffer

Add **500 µL** **of** AW1 **Qiagen Catalog #19081** to the All-Prep DNA spin column from step 5. Close the lid gently, and centrifuge for **00:00:15** at **10.000 rpm** . Discard the flow-through.

13.1 Reuse the spin column in step 15.

Note: Buffer AW1 is supplied as a concentrate. Ensure that ethanol is

added to Buffer AW1 before use.

14  2m

Buffer

Add **500 µL** **of** AW2 **Qiagen Catalog #19072** to the All-Prep DNA spin column. Close the lid gently, and centrifuge for **00:02:00** at full speed to wash the spin column membrane.

Note: Buffer AW2 is supplied as a concentrate. Ensure that ethanol is added to Buffer AW2 before use.

Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions or usage of resultant RNAs and or DNAs.

Note: After centrifugation, carefully remove the All-Prep DNA spin column from the collection tube. If the column contacts the flow-through, empty the collection tube and centrifuge the spin column again for **00:01:00** at full speed.

15 Place the All-Prep DNA spin column in a new 1.5mL collection tube. Add **50 µL** **of** 2m

Buffer

EB **Qiagen Catalog #19086** directly to the spin column membrane and close the lid. Incubate at room temperature (**15-25 °C**) for **00:01:00** , and then centrifuge for **00:01:00** at **10.000 rpm** to elute the DNA.

16 Repeat step 16 to elute further DNA.

Note: To achieve a higher DNA concentration, elute with 2 x **50 µL** Buffer EB. The final DNA yield, however, may be reduced.