Dec 08, 2020

Qiagen- AllPrep DNA/RNA/protein Mini Kit for tissue

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.bn9kmh4w

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ABSTRACT

The protocol is based on Qiagen-AllPrep DNA/RNA/Protein Mini kit for tissue

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Jin J, Robeson H, Fagan P, Orloff MS (2020) Association of *PARP1*-specific polymorphisms and haplotypes with non-small cell lung cancer subtypes. PLoS ONE 15(12): e0243509. doi: 10.1371/journal.pone.0243509

DOI

dx.doi.org/10.17504/protocols.io.bn9kmh4w

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

PROTOCOL CITATION

Heather Robeson, Jing Jin, Mohammed S. Orloff 2020. Qiagen- AllPrep DNA/RNA/protein Mini Kit for tissue. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bn9kmh4w

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243509

CREATED

Oct 30, 2020

LAST MODIFIED

Dec 08, 2020

PROTOCOL INTEGER ID

44044

MATERIALS TEXT

14.3 M β-mercaptoethanol (β-ME) mix with buffer RLT

Dithiothreitol (DTT)
Sterile, RNase-free pipet tips
Microcentrifuge (with rotor for 2 ml tubes)

96–100% ethanol
70% ethanol in water
Disposable gloves
liquid nitrogen

Equipment for sample disruption and homogenization

Trypsin and PBS
QIAshredder homogenizer, Blunt-ended needle and syringe
Mortar and pestle

The RNA content of the cell type
The DNA binding capacity of the AllPrep DNA spin column
The RNA binding capacity of the RNeasy spin column (100 µg RNA)

ABSTRACT

The protocol is based on Qiagen-AllPrep DNA/RNA/Protein Mini kit for tissue

BEFORE STARTING

-β-Mercaptoethanol (β-ME) is added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month.

-Dithiothreitol (DTT) must be added to Buffer ALO before use. Add 8 mg DTT per 1 ml Buffer ALO.

-Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

-Buffer RLT may form a precipitate during storage. If necessary, redissolve by warming, and then place at room temperature.

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-Preheat Buffer EB to 70°C to ensure optimal DNA elution.

Sample disruption and homogenization

1 Excise the tissue sample and determine the amount of tissue.

2 Homogenize the lysate in Buffer RLT
Disruption using a mortar and pestle. Homogenization using a QIAshredder homogenizer.

3 Centrifuge the lysate for 3 min at full speed. Remove the supernatant by pipetting and transfer the homogenized lysate to an AllPrep DNA spin column placed in a 2 ml collection tube.
Centrifuge for 30 s at ≥8000 x g (10,000 rpm).

4 Place the AllPrep DNA spin column in a new 2 ml collection tube, and store at room temperature (15–25°C) or at 4°C for later DNA purification. Use the flow-through for RNA purification.

Total RNA purification

5 To the flow-through, add 96–100% ethanol: either 250 µl (if 350 µl Buffer RLT was used) or 400 µl (if 600 µl Buffer RLT was used). Mix well by pipetting.

6 Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 15 s at ≥8000 x g (10,000 rpm). Transfer the flow-through to a 2 ml tube for protein purification.

7 Add 700 µl Buffer RW1 to the RNeasy spin column. Centrifuge for 15 s at ≥8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

8 Add 500 µl Buffer RPE to the RNeasy spin column. Centrifuge for 15 s at ≥8000 x g (10,000 rpm) to wash the spin column membrane. Discard the flow-through.

9 Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x g (10,000 rpm) to wash the spin column membrane.

10 Place the RNeasy spin column in a new 2 ml collection tube and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min.

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11 Place the RNeasy spin column in a new 1.5 ml collection tube. Add 30–50 µl RNase-free water to the spin column membrane. Centrifuge for 1 min at ≥ 8000 x g (10,000 rpm) to elute the RNA.

Genomic DNA purification

12 Add 500 µl Buffer AW1 to the AllPrep DNA spin column.
Centrifuge for 15 s at ≥8000 x g (10,000 rpm) to wash the spin column membrane.
Discard the flow-through.

13 Add 500 µl Buffer AW2 to the AllPrep DNA spin column.
Centrifuge for 2 min at full speed to wash the spin column membrane.

14 Place the AllPrep DNA spin column in a new 1.5 ml collection tube. Add 100 µl Buffer EB (preheated to 70°C) to the spin column membrane. Incubate at room temperature (15–25°C) for 2 min, and then centrifuge for 1 min at ≥8000 x g (10,000 rpm) to elute the DNA.

15 Repeat to elute further DNA.

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