Aug 25, 2020

Invitrogen PureLink® Genomic DNA Mini Kit

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1 Works for me This protocol is published without a DOI.

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PROTOCOL CITATION

Shlomi Abuchatzera Green 2020. Invitrogen PureLink® Genomic DNA Mini Kit. **protocols.io** https://protocols.io/view/invitrogen-purelink-genomic-dna-mini-kit-bivake2e

CREATED

Jul 22, 2020

LAST MODIFIED

Aug 25, 2020

PROTOCOL INTEGER ID

39554

BEFORE STARTING

Add 96–100% ethanol to PureLink® Genomic Wash Buffer 1 **and** PureLink® Genomic Wash Buffer 2 according to instructions on each label. Mix well.
Mark on the labels that ethanol is added.
Store both wash buffers with ethanol at room temperature.

Preparing lysates: Mammalian cells, tissues

1 Set a water bath or heat block at **55 °C**

2 **For adherent cells (≤5 × 10^6 cells):** remove the growth medium from the culture plate and harvest cells by trypisinization or a method of choice.

For suspension cells (≤5 × 106 cells): harvest cells and centrifuge the cells at 250 × g for 5 minutes to pellet cells. Remove the growth medium.

3

Resuspend the cells from Step 2 in **200 µl** PBS.

4 Add **20 µl** **Proteinase K** (supplied with the kit, stored in **4 °C**) to the sample.

5 

Add **20 µl** **RNase A** (supplied with the kit, stored in **4 °C**) to the sample, mix well by brief vortexing, and incubate at room temperature for 2 minutes **00:02:00** .

6 

Add **200 µl** **PureLink® Genomic Lysis/Binding Buffer** and mix well by vortexing to obtain a homogenous solution.

7 

Incubate at **55 °C** for 10 minutes **00:10:00** to promote protein digestion.

8 

Add **200 µl** **96–100% ethanol** to the lysate.
Mix well by vortexing for 5 seconds to yield a homogenous solution.

Binding DNA

9 Remove a **PureLink® Spin Column in a Collection Tube** from the package.

10 Add the lysate (~640 µL) to the PureLink® Spin Column.

11 

Centrifuge the column at 10,000 × g for 1 minute at room temperature.

10000 x g, Room temperature 00:01:00

If you are processing >200 µL starting material such as blood, buccal swabs, or Oragene™ preserved saliva, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same PureLink® Spin Column (above) and centrifuge at 10,000 × g for 1 minute.

12 Discard the collection tube and place the spin column into a clean PureLink® Collection Tube supplied with the kit.

Washing DNA

13 Add **500 µl Wash Buffer 1 prepared with ethanol** to the column.

14 Centrifuge column at room temperature at 10,000 × g for 1 minute.
10000 x g, Room temperature 00:01:00

15 Discard the collection tube and place the spin column into a clean PureLink® collection tube supplied with the kit.

16 Add **500 µl Wash Buffer 2 prepared with ethanol** to the column.

17 Centrifuge the column at maximum speed for 3 minutes at room temperature.
16000 x g, Room temperature 00:03:00

18 Discard collection tube.

Eluting DNA

19 Place the spin column in a sterile 1.5-mL microcentrifuge tube.

20 Add 25–200 µL of PureLink® Genomic Elution Buffer to the column.

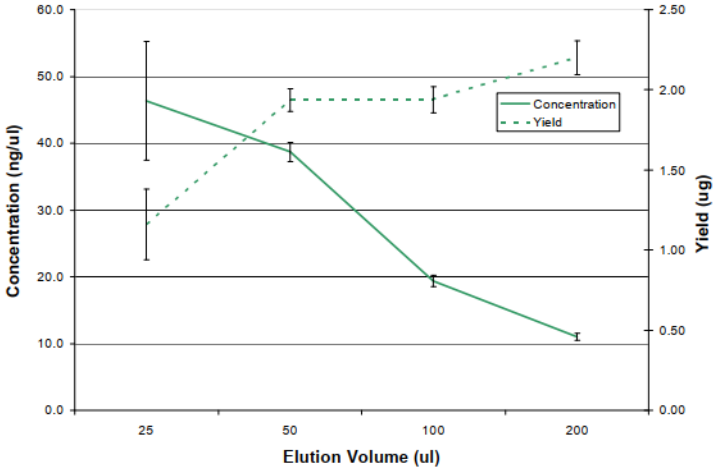

Figure Legend: Genomic DNA was purified from 100 µL blood samples with the PureLink® Genomic DNA Mini Kit using different elution volumes.

21 

Incubate at room temperature for 1 minute.

Room temperature **00:01:00**

22 

Centrifuge the column at maximum speed for 1 minute at room temperature.

16000 x g, Room temperature 00:01:00

The tube now contains purified genomic DNA.

23 

To recover more DNA, perform a second elution step using the same elution buffer volume as first elution in another sterile, 1.5-mL microcentrifuge tube.

24 Centrifuge the column at maximum speed for 1.5 minutes at room temperature.

16000 x g, Room temperature 00:01:30

25 The tube contains purified DNA. Remove and discard the column.

Storing DNA

26 Store the purified DNA at –20°C or use DNA for the desired downstream application.

27 For long-term storage, store the purified DNA in PureLink® Genomic Elution Buffer at –20°C as DNA stored in water is subject to acid hydrolysis.

28 To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.