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# 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

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## 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 ( $\leq 5 \times 10^6$  cells):** remove the growth medium from the culture plate and harvest cells by trypsinization or a method of choice.  
  
**For suspension cells ( $\leq 5 \times 10^6$  cells):** harvest cells and centrifuge the cells at  $250 \times g$  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.



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** .




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



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



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.



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.

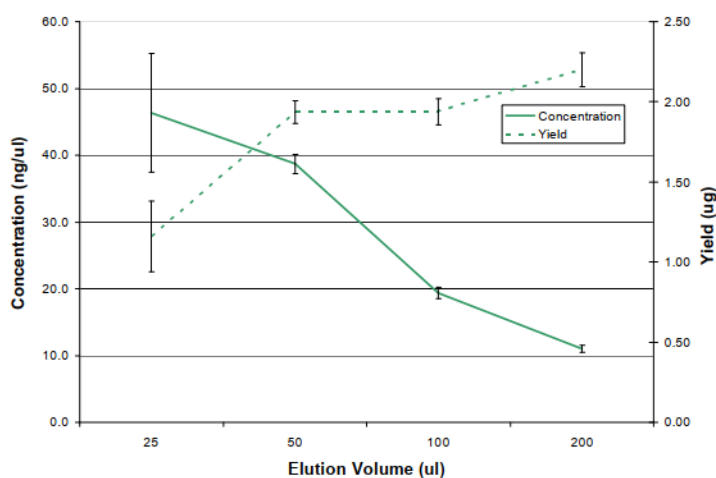
- 12 Discard the collection tube and place the spin column into a clean PureLink® Collection Tube supplied with the kit.

#### 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  $-20^{\circ}\text{C}$  or use DNA for the desired downstream application.

27 For long-term storage, store the purified DNA in PureLink® Genomic Elution Buffer at  $-20^{\circ}\text{C}$  as DNA stored in water is subject to acid hydrolysis.

28 To avoid repeated freezing and thawing of DNA, store the purified DNA at  $4^{\circ}\text{C}$  for immediate use or aliquot the DNA and store at  $-20^{\circ}\text{C}$  for long-term storage.