invitrogen™

# PureLink™ Genomic DNA Mini Kit

| Catalog No. | Quantity | Store at room temperature |
|---|---|---|
| K1820-00 | 10 Preps | |

## Contents and Storage

The components included with the PureLink™ Genomic DNA Mini Kit are listed below. Sufficient reagents are included to process 10 samples. Upon receipt, **store all components at room temperature**.

| Component | Quantity |
|---|---|
| PureLink™ Genomic Lysis/Binding Buffer | 2 ml |
| PureLink™ Genomic Digestion Buffer | 1.8 ml |
| PureLink™ Genomic Wash Buffer 1 | 2 ml |
| PureLink™ Genomic Wash Buffer 2 | 1.5 ml |
| PureLink™ Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA) | 2 ml |
| RNase A (20 mg/ml) in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA | 0.4 ml |
| Proteinase K (20 mg/ml) in storage buffer (proprietary) | 0.2 ml |
| PureLink™ Spin Columns with Collection Tubes | 10 |
| PureLink™ Collection Tubes (2.0 ml) | 20 |

## Description

The PureLink™ Genomic DNA Kits allow rapid and efficient purification of genomic DNA (gDNA). The kit is designed to efficiently isolate genomic DNA from mammalian cells and tissues, mouse/rat tail, blood samples, and bacteria. The kit includes robust buffers that allow simple lysis of cells and tissues with Proteinase K without the need for any mechanical lysis as well as allow efficient DNA binding to silica membrane. After preparing the lysate, genomic DNA is rapidly purified using a spin column based centrifugation procedure.

The isolated DNA is 20-50 kb in size and is suitable for PCR, restriction enzyme digestion, and Southern blotting.

## System Overview

The PureLink™ Genomic DNA Mini Kit is based on the selective binding of DNA to silica-based membrane in the presence of chaotropic salts. The lysate is prepared from a variety of starting materials such as tissues, cells, or blood. The cells or tissues are digested with Proteinase K at 55°C using an optimized digestion buffer formulation that aids in protein denaturation and enhances Proteinase K activity. Residual RNA is removed by RNase digestion prior to sample binding to the silica membrane.

The lysate is mixed with ethanol and PureLink™ Genomic Binding Buffer that allows high DNA binding to the silica-based membrane in the column. Impurities are removed by thorough washing with Wash Buffers 1 and 2. The genomic DNA is then eluted in low salt Elution Buffer.

## Specifications

| | |
|---|---|
| Starting Material: | Varies (see individual protocols) |
| Binding Capacity: | ~0.5 mg nucleic acid |
| Column Reservoir Capacity: | 800 µl |
| Collection Tube Capacity: | 2.0 ml (~700 µl without contacting column) |
| Centrifuge Compatibility: | Capable of centrifuging >10,000 x g |
| Elution Volume: | 25-200 µl |
| DNA Yield and Size: | DNA yield varies; DNA size is 20-50 kb |

## Caution

The Lysis/Binding Buffer and Wash Buffer 1 contain guanidine hydrochloride. Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers. Do not add bleach or acidic solutions directly to solutions containing guanidine hydrochloride or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids.

## Product Qualification

The PureLink™ Genomic DNA Kit is functionally qualified by isolating DNA from 1 ml overnight bacterial culture as described in this manual. The purified gDNA must have an $A_{260/280} \geq 1.70$ and agarose gel electrophoresis of the purified DNA must produce a single band above 12 kb. The protocol must yield >4 µg purified gDNA.

Part no. K1820.pps Rev. Date: 12 Feb 2007

## Important Guidelines

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no DNases are introduced into the sterile solutions of the kit
- Make sure all equipment that comes in contact with DNA is sterile including pipette tips and microcentrifuge tubes
- Do not vortex the samples for more than 5-10 seconds at each vortexing step to avoid extensive shearing of DNA
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples
- Perform all centrifugation steps at room temperature
- Be sure to perform the recommended wash steps to obtain the best results
- Handle all blood and tissue samples in compliance with established institutional guidelines and take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection) when handling blood and tissue samples. When processing blood and tissue samples, the eluates collected during wash steps contain biohazardous waste. Dispose the eluate and collection tubes appropriately as biohazardous waste.

## Elution Parameters

**Elution Buffer Volume**

The genomic DNA is eluted in 25-200 µl of PureLink™ Genomic Elution Buffer. You can change the volume of elution buffer to obtain genomic DNA in the desired final concentration. For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

**Elution Buffer**

The genomic DNA is eluted using PureLink™ Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA). Alternatively, Tris Buffer (10 mM Tris-HCl, pH 8.0-9.0) or sterile water can be used, if EDTA inhibits downstream reactions.

**Number of Elutions**

Using 50 µl and 100 µl elution buffer volume, the first elution recovers ~80% and 90% of bound genomic DNA, respectively. To maximize genomic DNA recovery, you may perform a second elution to recover the remaining 10-15% gDNA. Perform the second elution using the same volume of buffer used for first elution. To prevent dilution of the gDNA sample and also avoid contact of the spin column with the eluate, perform the two-elution steps using different tubes.

**Note:** Sufficient PureLink™ Genomic Elution Buffer is included to perform 2 x 100 µl elution per sample. If you wish to perform >2 x 100 µl elution per sample, you need to purchase additional PureLink™ Genomic Elution Buffer (catalog no. K1823-05) available separately.

## Materials Needed

- 96-100% ethanol
- Sample for DNA isolation (see appropriate protocol for recommended starting amount)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate
- Lysozyme and Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) for bacterial lysate
- Sterile, DNase-free microcentrifuge tubes
- Water baths or heat blocks and a microcentrifuge capable of centrifuging >10,000 x g

*Components supplied with the kit*

- PureLink™ Genomic Lysis/Binding Buffer
- PureLink™ Genomic Digestion Buffer
- Proteinase K (20 mg/ml) and RNase A (20 mg/ml)
- PureLink™ Genomic Wash Buffers 1 and 2
- PureLink™ Genomic Elution Buffer
- PureLink™ Spin Columns in Collection Tubes
- PureLink™ Collection Tubes

## Before Starting

- Make sure there is no precipitate visible in PureLink™ Genomic Digestion Buffer or PureLink™ Genomic Lysis/Binding Buffer. If any precipitate is visible in the buffers, warm the buffers at 37°C for 3-5 minutes and mix well to dissolve the precipitate before use.
- 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.

## Lysate Preparations

Protocols for preparing lysate from mammalian cells and tissues, mouse/rat tail, blood samples, and bacteria are described on pages 3-4. For additional lysate preparation protocols from yeast cells, FFPE (formalin-fixed paraffin-embedded) tissue, buccal swabs, and Oragene™ preserved saliva, refer to the PureLink™ Genomic DNA Kit manual available at www.invitrogen.com or contact Technical Support.

## Notes

- While processing multiple samples, you may prepare a master Digestion Buffer Mix when appropriate in the protocol by mixing 180 µl Digestion Buffer and 20 µl Proteinase K, and using 200 µl Digestion Buffer Mix per sample.
- While processing multiple samples, you may prepare a master Buffer/ethanol Mix when appropriate in the protocol by mixing 200 µl Lysis/Binding Buffer and 200 µl 96-100% ethanol, and using 400 µl Buffer/ethanol Mix per sample.

## Mammalian Cells and Blood Lysate Protocol

1. Set a water bath or heat block at 55°C.
2. Add 20 µl Proteinase K to a sterile microcentrifuge tube.
3. Process cells or blood samples:
   - For adherent cells (up to 5 x $10^6$ cells), remove the growth medium and harvest cells by trypisinization or a method of choice. Resuspend cells in 200 µl PBS.
   - For suspension cells (up to 5 x $10^6$ cells), harvest cells by centrifugation. Remove the growth medium. Resuspend cells in 200 µl PBS.
   - To a sterile microcentrifuge tube, add up to 200 µl fresh or frozen blood sample (if using <200 µl blood sample, adjust the sample volume to 200 µl using PBS). To process blood samples >200 µl and up to 1 ml, scale up all reagent volumes accordingly.

     **Note:** If you are processing >200 µl blood sample, you need to purchase additional PureLink™ Genomic Lysis/Binding Buffer (catalog no. K1823-02) and Proteinase K (catalog no. 25530-049) available separately. Visit www.invitrogen.com for details.
4. Transfer 200 µl cells or blood in PBS to the tube containing Proteinase K from Step 2.
5. Add 20 µl RNase A to the sample. Mix well by brief vortexing and incubate at room temperature for 2 minutes.
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 to promote protein digestion.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing to yield a homogenous solution.
9. Proceed immediately to **Purification Protocol**, next page.

## Mammalian Tissue and Mouse/Rat Tail Lysate Protocol

1. Set a water bath or heat block at 55°C.
2. Place up to 25 mg of minced mammalian tissue (up to 10 mg minced spleen tissue), or tail clip (1 cm mouse or 0.5 cm rat tail clips) into a sterile microcentrifuge tube:
3. Add 180 µl PureLink™ Genomic Digestion Buffer and 20 µl Proteinase K to the tube. Ensure the tissue is completely immersed in the buffer mix.
4. Incubate at 55°C with occasional vortexing until lysis is complete (1-4 hours). For mouse tails or larger tissue pieces, you may perform overnight digestion.
5. To remove any particulate materials, centrifuge the lysate at maximum speed for 3 minutes at room temperature. Transfer supernatant to a new, sterile microcentrifuge tube.
6. Add 20 µl RNase A to lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
7. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to yield a homogenous solution.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing to yield a homogenous solution.
9. Proceed immediately to **Purification Protocol**, next page.

## Blood Spots Protocol

1. Set a water bath or heat block at 55°C.
2. Place 2-5 punches of dried blood spot (2-3 mm in size) collected on paper such as FTA® card (Whatman) or S&S 903 in a sterile microcentrifuge tube.
3. Add 180 µl PureLink™ Genomic Digestion Buffer and 20 µl Proteinase K to the tube. Mix well by vortexing. Ensure the pieces are completely immersed in buffer.
4. Incubate at 55°C with occasional vortexing for 30 minutes.
5. Centrifuge the sample at maximum speed for 2-3 minutes at room temperature to pellet paper fibers. Transfer the sample to a new, sterile microcentrifuge tube.
6. Add 20 µl RNase A to lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
7. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing to yield a homogenous solution.
9. Proceed immediately to **Purification Protocol**, next page.

## Gram Negative Bacterial Cell Lysate Protocol

1. Set a water bath or heat block at 55°C.
2. Harvest up to 2 x $10^9$ Gram negative (~1 ml of overnight *E. coli* culture) by centrifugation.
3. Resuspend the cell pellet in 180 µl PureLink™ Genomic Digestion Buffer. Add 20 µl Proteinase K to lyse the cells. Mix well by brief vortexing.
4. Incubate the tube at 55°C with occasional vortexing until lysis is complete (30 minutes to up to 4 hours).
5. Add 20 µl RNase A to lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
6. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
7. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
8. Proceed immediately to **Purification Protocol**, below.

## Gram Positive Bacterial Cell Lysate Protocol

1. Set two water baths or heat blocks at 55°C and 37ºC, respectively.
2. Prepare Lysozyme Digestion Buffer (see recipe on page 2). To ~200 µl Lysozyme Digestion Buffer/sample, add **fresh** Lysozyme to obtain a final Lysozyme concentration of 20 mg/ml.
3. Harvest up to 2 x $10^9$ Gram positive cells by centrifugation.
4. Resuspend the cell pellet in 180 µl Lysozyme Digestion Buffer containing Lysozyme from Step 2. Mix well by brief vortexing.
5. Incubate at 37ºC for 30 minutes.
6. Add 20 µl Proteinase K. Mix well by brief vortexing.
7. Add 200 µl PureLink™ Genomic Lysis/Binding Buffer and mix well by vortexing to yield a homogenous solution.
8. Incubate at 55°C for 30 minutes.
9. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing to yield a homogenous solution.
10. Proceed immediately to **Purification Protocol**, below.

## Purification Protocol

The purification procedure is designed for purifying genomic DNA using a spin column-based centrifugation procedure in a total time of **10-15 minutes**.

1. Remove a PureLink™ Spin Column in a Collection Tube from the package.
2. Add the lysate (~640 µl) prepared with PureLink™ Genomic Lysis/Binding Buffer and ethanol to the spin column.
3. Centrifuge the column at 10,000 × g for 1 minute at room temperature.
   **Note:** If you are processing >200 µl starting material such as blood, 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 x g for 1 minute.
4. Discard the collection tube and place the spin column into a clean PureLink™ Collection Tube supplied with the kit.
5. Add 500 µl Wash Buffer 1 prepared with ethanol (page 2) to the column.
6. Centrifuge column at 10,000 × g for 1 minute at room temperature.
7. Discard the collection tube and place the spin column into a clean PureLink™ collection tube supplied with the kit.
8. Add 500 µl Wash Buffer 2 prepared with ethanol (page 2) to the column.
9. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
10. Place the spin column in a sterile 1.5-ml microcentrifuge tube.
11. Add 25-200 µl of PureLink™ Genomic Elution Buffer to the column. Choose the suitable elution volume for your needs.
12. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature.
    *The tube contains purified genomic DNA.*
13. To recover more DNA, perform a second elution step using the same elution buffer volume as first elution.
14. Centrifuge the column at maximum speed for 1.5 minutes at room temperature.
    *The tube contains purified DNA.* Remove and discard the column.
15. Use DNA for the desired downstream application or store the purified DNA at 4ºC (short-term) or -20°C (long-term).

## DNA Quantitation

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ Kits (visit www.invitrogen.com for details).