USER GUIDE

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PureLink® Genomic DNA Kits

For purification of genomic DNA

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Contents

Experienced Users' Mini Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® Genomic DNA Mini Kit. For more details, refer to this manual.

Step	Procedure		
Preparing lysates	Prepare the lysate using an appropriate sample preparation protocol as follows:		
	Sample	**Amount**	**Page no.**
	Mammalian cells, tissues and mouse tail	5×10^6 cells, ≤25 mg tissue (≤10 mg spleen), 0.5–1 cm tail	16
	Blood	≤1 mL non-nucleated blood (need additional reagents, see page 17)	17
		5–10 µL nucleated blood	
	Bacteria	$\leq 2 \times 10^9$ cells	18
	Yeast cells	$\leq 5 \times 10^7$ cells	19
	Buccal swab	Human buccal swab	19
	FFPE tissue	1–8 sections of 5–15 µm thick of about 20–50 mm^2 area	20
	Oragene™ preserved saliva	≤1 mL	21
Binding DNA	1. Remove a PureLink® Spin Column in a Collection Tube from the package. 2. **Load** the lysate (~640 µL) with Lysis/Binding Buffer and ethanol prepared as described on pages 16–21 to the PureLink® Spin Column. 3. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature. 4. Discard the collection tube and place the spin column into a new collection tube. 5. Proceed to **Washing DNA**.		
Washing DNA	1. **Wash** the column with 500 µL Wash Buffer 1 prepared with ethanol (page 23). 2. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature. Discard the collection tube and place column into a new collection tube. 3. **Wash** the column with 500 µL Wash Buffer 2 prepared with ethanol (page 23). 4. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard the collection tube. 5. Proceed to **Eluting DNA**.		
Eluting DNA	1. Place the spin column in a sterile 1.5-mL microcentrifuge tube. 2. **Elute** the DNA with 25–200 µL of PureLink® Genomic Elution Buffer. See **Elution Parameters** (page 13) to choose a suitable elution volume for your needs. 3. Incubate the column at room temperature for 1 minute. 4. Centrifuge the column at maximum speed for 1 minute at room temperature. *The tube contains purified DNA.* 5. If desired, perform a second elution to increase recovery which lowers the overall concentration. *The tube contains purified DNA.* Remove and discard the column. 6. Use the purified gDNA for the desired downstream application. Store the purified gDNA at 4ºC for short-term or –20°C for long-term storage.		

Experienced Users' 96 Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® 96 Genomic DNA Kit. For more details, refer to this manual.

Step	Procedure		
Preparing lysates	Prepare the lysate using an appropriate sample preparation protocol as follows:		
	Sample	**Amount**	**Page no.**
	Mammalian cells, tissues and mouse tail	5×10^6 cells, ≤25 mg tissue (≤10 mg spleen), 0.5–1 cm tail	27, 28
	Blood	≤200 µL non-nucleated blood	27
		5–10 µL nucleated blood	
	Bacteria	$\leq 2 \times 10^9$ cells	30
	Yeast cells	$\leq 5 \times 10^7$ cells	31
	FFPE tissue	1–8 sections of 5–15 µm thick of about 20–50 mm^2 area	32
	Oragene™ preserved Saliva	≤200 µL	33
	Buccal Swab	Human buccal swabs	33
Purification using centrifugation	1. Assemble the PureLink® gDNA Binding Plate onto a new or used 96 Deep Well Plate. 2. Transfer each lysate (~640 µL) to a well of the PureLink® gDNA Binding Plate using a multichannel pipettor. Cover any unused wells with Foil Tape. 3. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes. 4. Discard flow through and reassemble the Binding Plate onto the 96 Deep Well Plate. 5. Add 500 µL Wash Buffer 1 prepared with ethanol (page 35) into each well of the binding plate. 6. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes. 7. Discard the flow through and reassemble the plate stack. 8. Add 500 µL Wash Buffer 2 prepared with ethanol (page 35) into each well of the binding plate. 9. Centrifuge stacked plates at ≥2250 × *g* for 15 minutes to completely dry the membrane. **Note:** To ensure the complete drying of the membrane, do not seal the plate. 10. Discard flow through and reassemble the binding plate onto a **new** 96 Deep Well Plate. 11. Add 50–200 µL PureLink® Genomic Elution Buffer to the center of the membrane in each well and incubate the plate for 1 minute at room temperature. **Note:** Review **Elution Parameters** (page 13) to choose a suitable elution volume. 12. Centrifuge the stacked plates at ≥2250 × *g* for 3 minutes. *The purified gDNA is eluted in the Deep Well Plate.* 13. If desired, perform a second elution to increase recovery which lowers the overall concentration. 14. Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.		

Experienced Users' 96 Kit Procedure, Continued

Step	Procedure
Purification using vacuum manifold	1. Assemble the vacuum manifold as per the manufacturer's instructions. Brief instructions for assembling the PureLink® Vacuum Manifold with adaptors and PureLink® Genomic Wash Plate to prevent cross contamination are described on page 37. 2. Place the PureLink® gDNA Binding Plate onto the vacuum manifold. 3. Transfer the lysates (~640 µL) from each well of the Deep-Well Plate to a fresh well in the Binding Plate. Cover unused wells with Foil Tape. 4. Apply vacuum at room temperature until the lysate completely passes through the filter plate and release vacuum. 5. Add 1 mL Wash Buffer 1 prepared with ethanol (page 35) into each well of the Binding Plate. 6. Apply vacuum for 2 minutes at room temperature. Release vacuum. 7. Add 1 mL Wash Buffer 2 prepared with ethanol (page 35) into each well of the Binding Plate. 8. Apply vacuum for 2 minutes at room temperature. Release vacuum. 9. Disassemble the manifold to remove and discard the wash plate. Tap the Binding Plate on paper towels to remove any residual Wash Buffer from the nozzles. Reassemble the manifold with the Binding Plate. 10. Apply vacuum for 10 minutes at room temperature to dry the membrane. Release vacuum. 11. Disassemble the manifold to remove the waste tray. Discard the waste tray contents. 12. Assemble the vacuum manifold for elution as per the manufacturer's instructions. See page 38 for brief instructions on assembling various vacuum manifolds with different adaptors and elution plates. 13. Place the PureLink® gDNA Binding Plate onto the vacuum manifold. 14. Add 100–200 µL of PureLink® gDNA Elution Buffer to the center of the membrane and incubate the plate for 1 minute at room temperature. **Note:** Review **Elution Parameters** on page 13 to choose a suitable elution volume. 15. Apply vacuum for 2 minutes at room temperature. Release vacuum. Disassemble the vacuum manifold to remove the elution plate. *The purified gDNA is eluted into the elution plate.* 16. Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Kit Contents and Storage

Important

Before using this product, read and understand the information provided in the "Safety" appendix in this document.

Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog No.
PureLink® Genomic DNA Mini Kit	50 preps	K1820-01
	250 preps	K1820-02
PureLink® 96 Genomic DNA Kit	4 × 96 preps	K1821-04

Shipping and Storage

All components of the PureLink® Genomic DNA Kits are shipped at room temperature.

Upon receipt, store all components at room temperature.

Note: The Proteinase K solution and RNase A are stable for 1 year when stored at room temperature. For long-term storage (>1 year) or if room temperature is >25ºC, store the Proteinase K solution and RNase A at 4ºC.

PureLink® Genomic DNA Mini Kit Contents

The components included in the PureLink® Genomic DNA Mini Kits are listed in the following table.

Component	K1820-01	K1820-02
	50 preps	250 preps
PureLink® Genomic Lysis/Binding Buffer	10 mL	50 mL
PureLink® Genomic Digestion Buffer	9 mL	45 mL
PureLink® Genomic Wash Buffer 1	10 mL	50 mL
PureLink® Genomic Wash Buffer 2	7.5 mL	37.5 mL
PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	10 mL	50 mL
RNase A (20 mg/mL) in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA	1 mL	5 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	1 mL	5 mL
PureLink® Spin Columns with Collection Tubes	50 each	5 × 50 each
PureLink® Collection Tubes (2.0 mL)	100	5 × 100

PureLink® 96 Genomic DNA Kit Contents

The components included in the PureLink® 96 Genomic DNA Kit are listed in the following table.

Note: Some reagents in the kit may be provided in excess of the amount needed.

Component	Quantity
PureLink® Genomic Lysis/Binding Buffer	80 mL
PureLink® Genomic Digestion Buffer	70 mL
PureLink® Genomic Wash Buffer 1	2 × 100 mL
PureLink® Genomic Wash Buffer 2	2 × 75 mL
PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	160 mL
RNase A (20 mg/mL) in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA	8 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	8 mL
PureLink® Genomic DNA Binding Plate	4 plates
PureLink® Genomic DNA Wash Plate	4 plates
96 Deep Well Plate	2 × 6 plates
Foil Tape	20/pack

Introduction

Overview

Introduction

The PureLink® Genomic DNA Kits allow rapid and efficient purification of genomic DNA. The kit is designed to efficiently isolate genomic DNA from mammalian cells and tissues, mouse/rat tail, blood samples, buccal swabs, bacteria, yeast, FFPE (formalin-fixed paraffin-embedded) tissue, and Oragene™ preserved saliva. After preparing the lysates, the DNA is rapidly purified from lysates using a spin column based centrifugation procedure or high throughput isolation using 96-well plates with a vacuum manifold or automated liquid handling workstations.

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 Kits are 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. Any residual RNA is removed by digestion with RNase A prior to binding samples to the silica membrane.

The lysate is mixed with ethanol and PureLink® Genomic Binding Buffer that allows high DNA binding PureLink® Spin Column (Mini Kit) or Binding Plate (96 Kit). The DNA binds to the silica-based membrane in the column or plate and impurities are removed by thorough washing with Wash Buffers. The genomic DNA is then eluted in low salt Elution Buffer.

Advantages

The advantages of using PureLink® Genomic DNA Kits are:

- Rapid and efficient purification of genomic DNA from a variety of samples such as mammalian cells and tissue, blood samples, mouse tails, buccal swabs, bacteria, yeast, FFPE tissue, and Oragene™ preserved saliva
- Designed to rapidly purify high-quality DNA using spin column or 96-well plate format
- Automation using standard robotic systems (96 kit) with no sample cross contamination
- Simple lysis of cells and tissues with Proteinase K without the need for any mechanical lysis
- Minimal contamination from RNA
- Reliable performance of the purified DNA in PCR, restriction enzyme digestion, and Southern blotting

Mini Kit Specifications

Starting Material:	Varies (see page 12)
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 × *g*
Elution Volume:	25–200 µL
DNA Yield:	Varies (see page 40)
DNA Size:	20–50 kb

96 Kit Specifications

Dimensions:	Standard SBS (Society for Biomolecules Screening) footprint
Starting Material:	Varies (see page 12)
Binding Capacity:	~0.5 mg nucleic acid
Binding Plate Capacity:	1 mL
Deep-Well Plate Capacity:	1.0 mL (0.75 mL without contacting nozzles)
Centrifuge Compatibility:	Capable of centrifuging at ≥2,250 × *g* Bucket depth 5.75 cm
Elution Volume:	50–200 µL
DNA Yield:	Varies
DNA Size:	20–50 kb

Methods

General Guidelines

Introduction

General guidelines for using the PureLink® Genomic DNA Kits are described in the following sections. Review this section before starting the purification procedure.

Choose the appropriate purification protocol based on the type of kit you have purchased:

Kit	Page no.
PureLink® Genomic DNA Mini Kits (K1820-01, K1820-02)	14
PureLink® 96 Genomic DNA Kit (K1821-04)	25

To obtain high-quality genomic DNA, follow the guidelines recommended on page 11.

Follow the listed recommendations to obtain the best results:

- 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

RNase A Digestion

RNase A digestion is performed during sample preparation to degrade RNA present in the sample and minimize RNA contamination in the purified DNA sample. RNA contamination also inflates the DNA content measured at 260 nm.

RNase A is supplied with the kit and an RNase digestion step is included during sample preparation protocols.

If RNA content of the sample is minimal (e.g., mouse tail) and RNA contamination *does not* interfere with any downstream applications of the purified DNA, you may omit the RNase digestion step during sample preparation.

Proteinase K Digestion

The Proteinase K is used for efficient lysis of tissues/cells. Proteinase K digestion is performed using an optimized buffer formulation, PureLink® Genomic Digestion Buffer, for optimal enzymatic activity.

Sample Amount

There are different protocols for preparing lysates depending on the starting material (sample). Based on your sample, choose an appropriate lysate preparation protocol from the following table.

The PureLink® Genomic DNA Kits are suitable for isolating genomic DNA from a variety of samples using the recommended sample amount (see table below).

Note: If you start with less amount of sample, the yield of DNA may also be lower.

To obtain high yield of DNA and minimize DNA degradation, collect the sample and proceed immediately to sample preparation or freeze the sample in liquid nitrogen immediately after collection.

Sample	Amount
Mammalian cells	5×10^6 cells (suspension or adherent cells)
Mammalian tissues	≤25 mg (≤10 mg for spleen)
Mouse or rat tail	1 cm (mouse); 0.5 cm (rat)
Buccal swab	Human buccal swab
Nonnucleated whole blood (e.g., human, mouse)	≤200 µL (single pass) ≤1 mL (multiple pass, Mini Kit only)
Nucleated whole blood (e.g., bird)	5–10 µL
Blood spot on paper	2–5 punches (2–3 mm in size)
Gram negative bacteria (e.g., *E. coli*)	$\leq 2 \times 10^9$ cells
Gram positive bacteria (e.g., Bacillus)	$\leq 2 \times 10^9$ cells
Yeast cells	$\leq 5 \times 10^7$ cells
FFPE tissue	1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2.
Oragene™ preserved saliva	≤4 mL (Mini Kit); ≤200 µL (96 Kit)

Whole Blood Sample

The PureLink® Genomic DNA Kits are designed to purify genomic DNA from the following whole blood samples:

- Fresh or frozen whole blood
- Fresh or frozen whole blood collected in the presence of anti-coagulants such as EDTA or citrate
- Dried blood spots on paper such as FTA® card (Whatman) or S&S 903.

Elution Parameters

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.

Elution Buffer Volume

The genomic DNA is eluted in 25–200 µL (Mini Kit) or 50–200 µL (96 Kit) of PureLink® Genomic Elution Buffer. You can change the volume of elution buffer to obtain genomic DNA in the desired final concentration. Use the graph shown below to determine the most appropriate elution conditions for your application. For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

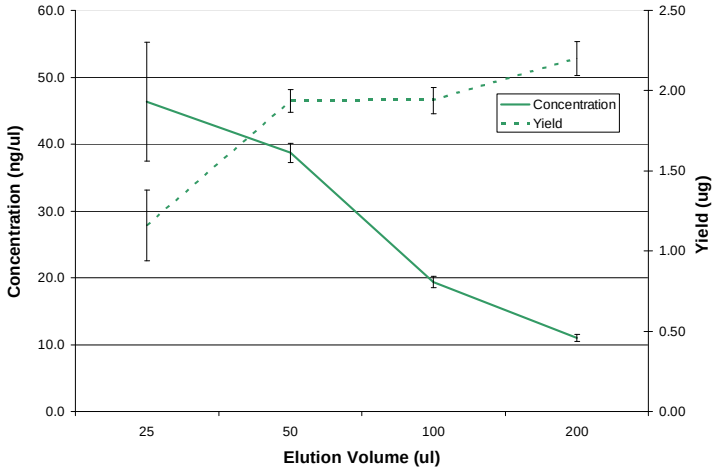


Figure Legend: Genomic DNA was purified from 100 µL blood samples with the PureLink® Genomic DNA Mini Kit using different elution volumes.

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. To prevent dilution of the gDNA sample perform the second elution using the same volume of buffer used for first elution. Avoid contact of the spin column with the eluate by using different tubes for the two-elution steps.

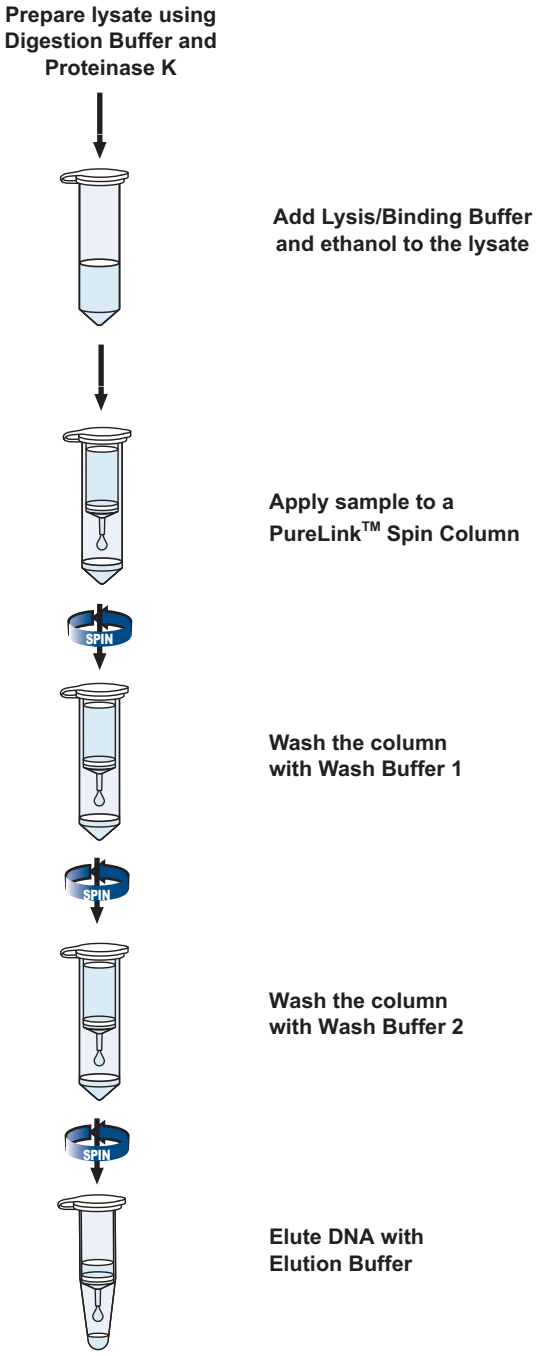
Note: Sufficient PureLink® Genomic Elution Buffer is included to perform up to 2 × 100 µL elution per sample. If you wish to perform >2 × 100 µL elution per sample, you need to purchase additional PureLink® Genomic Elution Buffer (page 43) available separately.

Purification Procedure Using Mini Kit

Experimental Overview

Introduction

The procedure for purifying genomic DNA using the PureLink® Genomic DNA Mini Kits is illustrated in the following flow chart.



Preparing Lysates–Mini Kit

Introduction

Instructions for preparing lysates from mammalian cells and tissues, mouse tail, buccal swabs, blood, bacteria, yeast, FFPE tissues, and Oragene™ preserved saliva are described in the following sections.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 11.

Important

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.

Materials Needed

Components supplied by the user

- 96–100% ethanol
- Sample for DNA isolation (see page 12 for recommended starting amount)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate (page 43)
- Lysozyme and Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) for bacterial cell lysate
- Zymolase Buffer (1 M sorbitol, 10 mM sodium EDTA, 14 mM β-mercaptoethanol) and Zymolase (lyticase) enzyme for yeast lysate
- CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) or xylene for FFPE tissue
- 3 M sodium acetate (pH 5–5.5) and 2.8 mL isopropanol for Oragene™ samples
- Sterile, DNase–free microcentrifuge tubes
- Water baths or heat blocks

Components supplied with the Kit

- PureLink® Genomic Lysis/Binding Buffer
- PureLink® Genomic Digestion Buffer
- Proteinase K (20 mg/mL)
- RNase A (20 mg/mL)

Preparing Lysates–Mini Kit, Continued

Mammalian Cells Lysate

Use the following protocol to prepare lysate from mammalian cells.

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 × 10^6 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) to the sample.
5. Add 20 µL RNase A (supplied with the kit) 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 for 5 seconds to yield a homogenous solution.
9. Proceed immediately to **Binding DNA** (page 23).

Mammalian Tissue and Mouse/Rat Tail Lysate

Use the following protocol to prepare lysate from mammalian tissues and mouse/rat tails.

1. Set a water bath or heat block at 55°C.
2. Place the following amount of mammalian tissue or tail into a sterile microcentrifuge tube:
   - ≤25 mg of minced mammalian tissue
   - ≤10 mg minced spleen tissue
   - 1 cm mouse or 0.5 cm rat tail clip
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to the tube. Ensure the tissue is completely immersed in the buffer mix.

   Note: When processing multiple samples, prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
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 (supplied in the kit) to the 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.
8. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds.

   Note: When processing multiple samples, prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol per sample.
9. Proceed immediately to **Binding DNA** (page 23).

Blood Lysate

Use the following protocol to prepare lysate from blood samples (nucleated or nonnucleated).

Note: If you are processing >200 µL blood sample, you need to purchase additional PureLink® Genomic Lysis/Binding Buffer and Proteinase K (page 43).

1. Set a water bath or heat block at 55°C.
2. To a sterile microcentrifuge tube, add ≤200 µL fresh or frozen blood sample (if using <200 µL blood sample, adjust the sample volume to 200 µL using PBS).

   For processing blood samples >200 µL and ≤1 mL, scale up all reagent volumes accordingly.
3. Add 20 µL Proteinase K (supplied with the kit) to the sample.
4. Add 20 µL RNase A (supplied with the kit) to the sample, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
5. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
6. Incubate at 55°C for 10 minutes to promote protein digestion.
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 **Binding DNA** (page 23).

Blood Spots

Use the following protocol to prepare lysate from dried blood spots.

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) in a sterile microcentrifuge tube.
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) 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 clean, sterile microcentrifuge tube.
6. Add 20 µL RNase A (supplied in the kit) to the 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 for 5 seconds to yield a homogenous solution.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Binding DNA** (page 23).

Gram Negative Bacterial Cell Lysate

Use the following protocol to prepare Gram negative bacterial cell lysate.

1. Set a water bath or heat block at 55°C.
2. Harvest up to 2×10^9 Gram negative (~1 mL of overnight *E. coli* culture) by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
3. Resuspend the cell pellet in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit) 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 (supplied with the kit) to the 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.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
8. Proceed to **Binding DNA** (page 23).

Gram Positive Bacterial Cell Lysate

Use the following protocol to prepare Gram positive bacterial cell lysate.

1. Set two water baths or heat blocks at 37ºC and 55°C, respectively.
2. Prepare Lysozyme Digestion Buffer (see recipe on page 15). To ~200 µL Lysozyme Digestion Buffer/sample, add **fresh** Lysozyme to obtain a final Lysozyme concentration of 20 mg/mL.
3. Harvest up to 2×10^9 Gram positive cells by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
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 (supplied with the kit). Mix well by brief vortexing.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by brief vortexing.
8. Incubate at 55°C for 30 minutes.
9. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
10. Proceed to **Binding DNA** (page 23).

Yeast Cells Lysate

Use the following protocol to prepare lysate from yeast cells.

1. Set 2 water baths or heat blocks at 37°C and 55°C, respectively.
2. Prepare **fresh** Zymolase Buffer (see page 15). You need 500 µL buffer per sample.
3. Harvest up to 5×10^7 yeast cells by centrifugation. If you are using a frozen cell pellet, proceed to Step 4.
4. Resuspend the cell pellet in 500 µL Zymolase Buffer. Add 15 units Zymolase (lyticase) enzyme and incubate at 37°C for 1 hour to generate spheroplasts.
5. Centrifuge at 3000 × *g* for 10 minutes at room temperature to pellet the spheroplasts. Discard the supernatant.
6. Resuspend the spheroplasts in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit). Mix well by brief vortexing.
7. Incubate at 55°C for 45 minutes.
8. Add 20 µL RNase A (supplied in the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
9. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by brief vortexing to obtain a homogenous solution.
10. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
11. Proceed immediately to **Binding DNA** (page 23).

Human Buccal Swab Lysate

Use the following protocol to prepare lysate from human buccal cell swabs.

1. Set a water bath or heat block at 55°C.
2. Place the buccal swab in a sterile, 2-mL microcentrifuge tube. Add 400 µL (for cotton and Dacron swab) or 600 µL (for Omni Swab) PBS to the sample.
3. Add 20 µL Proteinase K into a sterile microcentrifuge tube capable of holding three times the volume of lysate (for example, if you plan to process 600 µL lysate, use a microcentrifuge tube capable of holding 1800 µL).
4. Transfer 200–600 µL swab lysate to the microcentrifuge tube containing Proteinase K (Step 3). Mix well by pipetting.
5. Add an equal volume of PureLink® Genomic Lysis/Binding Buffer to the lysate and mix well by brief vortexing.

   For example, if you are processing 200 µL lysate, add 200 µL PureLink® Genomic Lysis/Binding Buffer.
6. Incubate at 55°C for at least 10 minutes.
7. Centrifuge briefly to collect any lysate from the tube caps.
8. Add 200 µL 96–100% ethanol to the tube. Mix well by vortexing for 5 seconds to yield a homogenous solution.
9. Proceed immediately to **Binding DNA** (page 23).

FFPE Tissue Lysate

Prepare lysate from FFPE (formalin-fixed, paraffin-embedded) tissue as described below.

1. Set 2 water baths or heat blocks at 37ºC and 50°C, respectively.
2. Place 1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2 (no more than 20 mg tissue) in a sterile microcentrifuge tube.
3. Add 1 mL CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) to the sample and vortex vigorously for a few seconds.

   CitriSolv™ Clearing Agent is a biodegradable alternative to xylene for paraffin extraction.

   Note: You may also use xylene instead of CitriSolv™. Use appropriate precautions while using xylene and dispose of xylene in compliance with established institutional guidelines.
4. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
5. Add 1 mL 96–100% ethanol and vortex to resuspend the tissue pellet.
6. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
7. Repeat ethanol extraction (Steps 5–6) once more.
8. Incubate the tubes with lid open at 37ºC for 5–10 minutes to evaporate any residual ethanol.
9. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit). Mix well by brief vortexing.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
10. Incubate at 50°C for 3 hours to overnight.
11. Centrifuge the lysate at maximum speed for 3 minutes at room temperature to remove any particulate materials. Transfer lysate to a new, sterile microcentrifuge tube.
12. Add 20 µL RNase A (supplied in the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
13. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by brief vortexing.
14. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
15. Proceed immediately to **Binding DNA** (page 23).

Oragene™ Preserved Saliva

Process Oragene™ preserved saliva as described below.

Note: If you are processing >200 µL Oragene™ sample, you need to purchase additional PureLink® Genomic Lysis/Binding Buffer and Proteinase K (page 43).

Saliva Collection

Collect and preserve saliva as described by the Oragene™ device manufacturer. If the saliva sample is collected immediately prior to purification, incubate the sample at 50ºC for 1 hour before starting the protocol. Otherwise, an overnight incubation at room temperature in the Oragene™ device is sufficient to release and preserve genomic DNA.

Up to 1 mL Oragene™ Sample

1. Set a water bath or heat block at 55°C.
2. Transfer 1 volume of saliva mixture from Oragene™ self-collection device into an appropriate tube and mix with 1 volume of PureLink® Genomic Lysis/Binding Buffer. Choose an appropriate tube that can hold three times your sample volume to allow for reagent additions.

   For example, to 200 µL Oragene™ sample, add 200 µL PureLink® Genomic Lysis/Binding Buffer.
3. Incubate at 55ºC for 10 minutes.
4. Add 1 sample volume of 96–100% ethanol and mix well by brief vortexing.

   For example, if you used 200 µL Oragene™ sample, add 200 µL 96–100% ethanol.
5. Proceed immediately to **Binding DNA** (page 23).

4 mL Oragene™ Sample

1. Set a water bath or heat block at 55–65°C.
2. To the entire ~4 mL Oragene™ sample, add 400 µL 3 M sodium acetate (pH 5–5.5) and 2.8 mL isopropanol. Mix well by brief vortexing.
3. Centrifuge at >12,000 × *g* for 10 minutes at room temperature. Discard the supernatant.
4. Resuspend the pellet in 300 µL PBS (or 10 mM Tris, pH 8.0), and add 300 µL PureLink® Genomic Lysis/Binding Buffer.
5. Incubate at 55–65ºC for 5–10 minutes to solubilize the pellet containing cell debris and nucleic acid.
6. Add 300 µL 96–100% ethanol and mix well by brief vortexing.
7. Proceed immediately to **Binding DNA** (page 23).

Guidelines for Lysate Protocol Development

If none of the lysate preparation protocols described in this manual match the type or size of your sample, use the following guidelines to develop your own lysate preparation protocol.

- Lyse the sample using the PureLink® Genomic Digestion Buffer and Proteinase K supplied with the kit or use specialized lysis buffer or protocols to perform lysis. You may need to optimize lysis conditions prior to DNA purification to obtain the best results for your specific sample.
- Mix the sample with PureLink® Genomic Binding Buffer and 96–100% ethanol prior to loading the sample onto the column. Always maintain a ratio of 1:1:1 for Sample/Digestion Buffer:Binding Buffer:Ethanol to obtain optimal DNA binding.

A general protocol for lysate preparation can be as follows:

1. For cells, harvest cells and resuspend cell pellet in 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K. Incubate at 55°C until lysis is complete.

   For tissues, start with a small amount of minced tissue and add 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K to the sample and mix well. Incubate at 55°C until lysis is complete.

   Based on the results obtained using this lysis protocol, you may need to optimize the lysis protocol using different buffers or increasing the amount and time of Proteinase K digestion.

   If you already have a lysate, proceed to Step 2.
2. Add 20 µL RNase A (supplied with the kit). Incubate at room temperature for 2 minutes.
3. Centrifuge the lysate at maximum speed for 5 minutes at room temperature to remove any particulate material, if needed.
4. Transfer the supernatant to a fresh microcentrifuge tube. Add 200 µL PureLink® Genomic Binding Buffer supplied with the kit to the lysate. Mix well by vortexing to yield a homogenous solution.
5. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
6. Proceed to **Binding DNA**, page 23.

Purification Procedure Using Spin Columns

Introduction

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

Materials Needed

Components supplied by the user

- Lysates prepared as described on pages 16–21
- Sterile, DNase-free 1.5-mL microcentrifuge tubes for elution
- Microcentrifuge capable of centrifuging >10,000 × g
- Optional: sterile water, pH 7.0–8.5, if you are using water for elution

Components supplied with the Kit

- PureLink® Genomic Wash Buffers 1 and 2
- PureLink® Genomic Elution Buffer
- PureLink® Spin Columns in Collection Tubes
- PureLink® Collection Tubes

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Review **Elution Parameters** on page 13 to determine the suitable elution volume for your requirements
- Perform a 1 minute incubation step with PureLink® Genomic Elution Buffer
- Be sure to perform the recommended wash steps to obtain the best results
- If you are using water for elution, always use sterile water, pH 7.0–8.5

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.

Binding DNA

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 PureLink® 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, 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.
4. Discard the collection tube and place the spin column into a clean PureLink® Collection Tube supplied with the kit.
5. Proceed to **Washing DNA**, page 24.

Purification Procedure Using Spin Columns, Continued

Washing DNA

1. Add 500 µL Wash Buffer 1 prepared with ethanol (page 23) to the column.
2. Centrifuge column at room temperature at 10,000 × *g* for 1 minute.
3. Discard the collection tube and place the spin column into a clean PureLink® collection tube supplied with the kit.
4. Add 500 µL Wash Buffer 2 prepared with ethanol (page 23) to the column.
5. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
6. Proceed to **Eluting DNA**.

Eluting DNA

1. Place the spin column in a sterile 1.5-mL microcentrifuge tube.
2. Add 25–200 µL of PureLink® Genomic Elution Buffer to the column. See **Elution Parameters** (page 13) to choose the suitable elution volume for your needs.
3. 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.*
4. 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.
5. Centrifuge the column at maximum speed for 1.5 minutes at room temperature.

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

Storing DNA

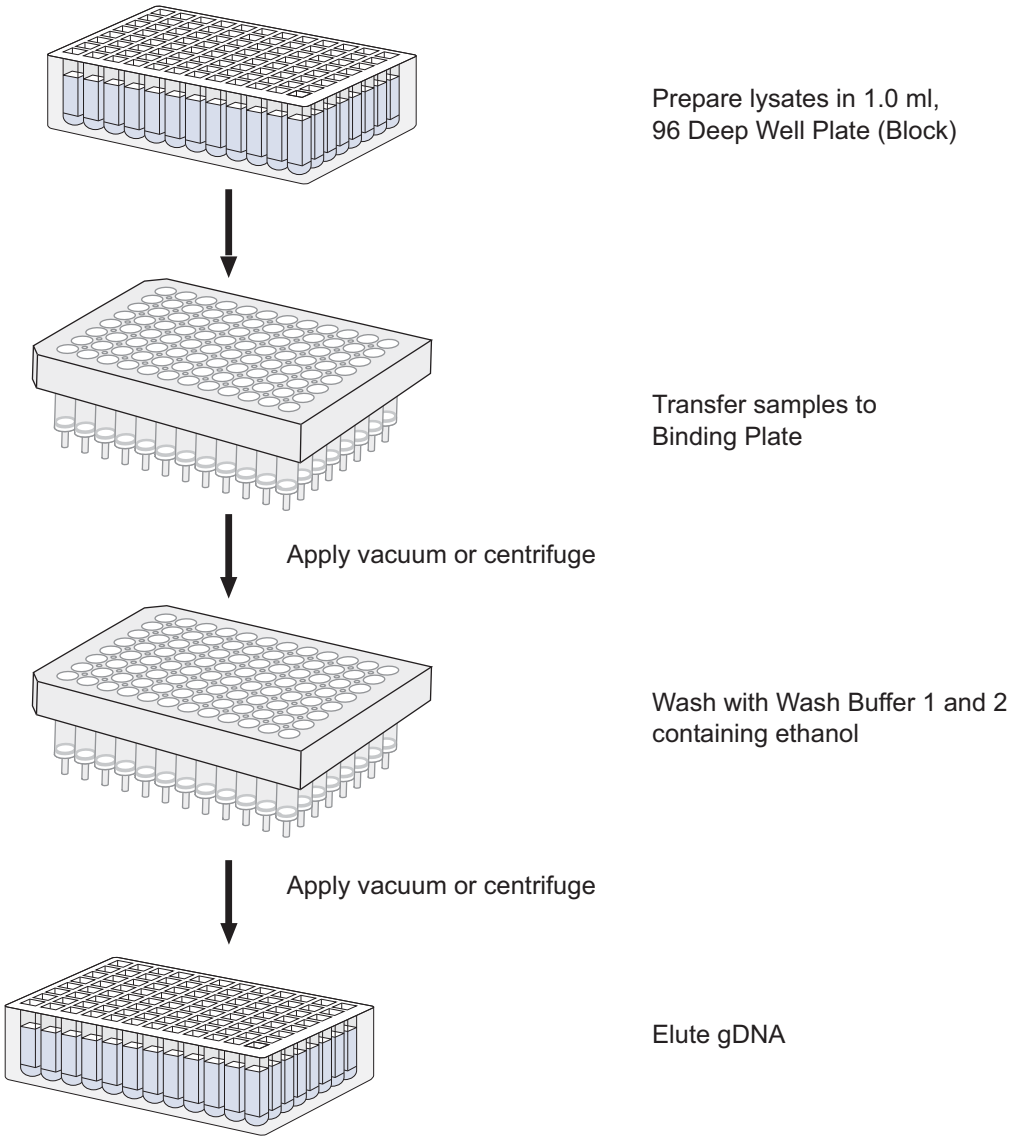
- Store the purified DNA at –20°C or use DNA for the desired downstream application.
- For long-term storage, store the purified DNA in PureLink® Genomic Elution Buffer at –20°C as DNA stored in water is subject to acid hydrolysis.
- To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

Purification Procedure Using 96 Kit

Experimental Overview

Introduction

The procedure for purifying genomic DNA using the PureLink® 96 Genomic DNA Kit is illustrated in the following flow chart.



Preparing Lysates–96 Kit

Introduction

Instructions for preparing lysates from mammalian cells and tissues, mouse tail, blood, buccal swabs, bacteria, yeast, FFPE tissues, and Oragene™ preserved saliva are described in the following sections.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 11.

Important

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.

Materials Needed

Components supplied by the user

- 96–100% ethanol
- Sample for DNA isolation (see page 12 for recommended starting amount)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate (page 43)
- Lysozyme and Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) for bacterial cell lysate
- Zymolase Buffer (1 M sorbitol, 10 mM sodium EDTA, 14 mM β-mercaptoethanol) and Zymolase (lyticase) enzyme for yeast lysate
- CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) or xylene for FFPE tissue
- 3 M sodium acetate (pH 5–5.5) and 2.8 mL isopropanol for Oragene™ samples
- Water baths or heat blocks

Components supplied with the Kit

- PureLink® Genomic Lysis/Binding Buffer
- PureLink® Genomic Digestion Buffer
- Proteinase K (20 mg/mL)
- RNase A (20 mg/mL)
- 96 Deep Well Plates
- Foil Tape

Note

Do not reuse the Foil Tape during lysate preparation steps. After use, discard the Foil Tape and use a fresh Foil Tape for the next step. Sufficient Foil Tape is included in the kit. Additional Foil Tape is also available separately, see page 43 for ordering information.

Mammalian Cells and Blood Lysate

Use the following protocol to prepare lysate from mammalian cells and blood samples.

1. Set a water bath or heat block at 55°C.
2. Add 20 µL Proteinase K (supplied with the kit) to each well of a 96 Deep Well Plate.
3. Process cells or blood samples:
   - 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. Resuspend the cells in 200 µL PBS.
   - For suspension cells (≤5 × 10^6 cells), harvest cells by centrifugation at 250 × *g* for 5 minutes. Remove the growth medium. Resuspend the cells in 200 µL PBS.
   - To a sterile microtiter plate, 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).
4. Transfer 200 µL cells or blood in PBS to each well of a 96 Deep Well Plate containing Proteinase K from Step 2.
5. Add 20 µL RNase A (supplied with the kit) to the sample. Seal the plate with Foil Tape. Mix well by brief vortexing, and incubate at room temperature for 2 minutes.
6. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and seal the plate with Foil Tape. Mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at 55°C for 10 minutes to promote protein digestion.
8. Add 200 µL 96–100% ethanol to the lysate and seal the plate with Foil Tape. Mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Mammalian Tissue and Mouse/Rat Tail Lysate

Use the following protocol to prepare lysate from mammalian tissues and mouse/rat tails.

1. Set a water bath or heat block at 55°C.
2. Place the following amount of mammalian tissue or tail into each well of a 96 Deep Well Plate:
   - Up to 25 mg of minced mammalian tissue
   - Up to 10 mg minced spleen tissue
   - 1 cm mouse or 0.5 cm rat tail clip
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to each well of the 96 Deep Well Plate. Ensure the tissue is completely immersed in the buffer mix. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
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. Centrifuge the lysate at maximum speed for 3 minutes at room temperature to remove any particulate materials.
6. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and seal the plate with Foil Tape. Mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
8. Add 200 µL 96–100% ethanol to the lysate and seal the plate with Foil Tape. Mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Blood Spots

Use the following protocol to prepare lysate from dried blood spots.

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) in a well of a 96 Deep Well Plate.
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to each well. Ensure the pieces are completely immersed in buffer. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
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 samples to a clean, 96 Deep Well Plate.
6. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal plate with the Foil Tape and mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
8. Add 200 µL 96–100% ethanol to the lysate. Seal plate with the Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Gram Negative Bacterial Cell Lysate

Use the following protocol to prepare Gram negative bacterial cell lysate.

1. Set a water bath or heat block at 55°C.
2. Harvest up to 2 × 10^9 Gram negative (~1 mL of overnight *E. coli* culture) bacteria in a 96 Deep Well Plate by centrifugation at ≥2250 × *g* for 10 minutes.
3. Resuspend the cell pellet in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit) to lyse the cells. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
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 (supplied with the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
6. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
8. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Gram Positive Bacterial Cell Lysate

Use the following protocol to prepare Gram positive bacterial cell lysate.

1. Set two water baths or heat blocks at 37ºC and 55°C, respectively.
2. Prepare Lysozyme Digestion Buffer (see recipe on page 26). 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 × 10^9 Gram positive cells in a 96 Deep Well Plate by centrifugation at ≥2250 × *g* for 10 minutes.
4. Resuspend the cell pellet in 180 µL Lysozyme Digestion Buffer with Lysozyme from Step 2. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
5. Incubate at 37ºC for 30 minutes.
6. Add 20 µL Proteinase K and 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at 55°C for 30 minutes.
8. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Yeast Cells Lysate

Use the following protocol to prepare lysate from yeast cells.

1. Set 2 water baths or heat blocks at 37°C and 55°C, respectively.
2. Prepare **fresh** Zymolase Buffer (see recipe on page 26). You need 500 µL buffer per sample.
3. Harvest up to 5×10^7 yeast cells by centrifugation in a 96 Deep Well Plate.
4. Resuspend the cell pellet in 500 µL Zymolase Buffer. Add 15 units Zymolase (lyticase) enzyme and incubate at 37°C for 1 hour to generate spheroplasts.
5. Centrifuge at 3000 × *g* for 10 minutes at room temperature to pellet the spheroplasts.
6. Resuspend the spheroplasts in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit). Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at 55°C for 45 minutes.
8. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
9. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
10. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
11. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

FFPE Tissue Lysate

Use the following protocol to prepare lysates from FFPE (formalin-fixed, paraffin-embedded) tissue.

1. Set 2 water baths or heat blocks at 37ºC and 50°C, respectively.
2. Place 1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2 (no more than 20 mg tissue) into each well of a 96 Deep Well Plate.
3. Add 1 mL CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) to the sample. Seal the plate with Foil Tape and vortex vigorously for a few seconds.

   CitriSolv™ Clearing Agent is a biodegradable alternative to xylene for paraffin extraction.

   Note: You may also use xylene instead of CitriSolv™. Use appropriate precautions while using xylene and dispose of xylene in compliance with established institutional guidelines.
4. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
5. Add 1 mL 96–100% ethanol. Seal the plate with Foil Tape and vortex to resuspend the tissue pellet.
6. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
7. Repeat ethanol extraction (Steps 5–6) once more.
8. Incubate the plate **without the seal** at 37ºC for 5–10 minutes to evaporate any residual ethanol.
9. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit). Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
10. Incubate at 50°C for 3 hours to overnight.
11. Centrifuge the lysate at maximum speed for 3 minutes at room temperature to remove any particulate materials.
12. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
13. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
14. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
15. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Human Buccal Swab Lysate

Use the following protocol to prepare lysate from human buccal cell swabs.

1. Set a water bath or heat block at 55°C.
2. Place the buccal swab in a sterile, 2 mL microcentrifuge tube. Add 400 µL (for cotton and Dacron swab) or 600 µL (for Omni Swab) PBS to the sample.
3. Add 20 µL Proteinase K into the wells of a 96 Deep Well Plate.
4. Transfer 200 µL swab lysate to 96 Deep Well Plate containing Proteinase K (Step 3).
5. Add 200 µL PureLink® Genomic Lysis/Binding Buffer to the lysate. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
6. Incubate at 55°C for at least 10 minutes. Keep the plate covered with Foil Tape during the incubation.
7. Centrifuge briefly to collect any lysate from the Foil Tape.
8. Add 200 µL 96–100% ethanol to the tube. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Oragene™ Preserved Saliva

Process up to 200 µL Oragene™ preserved saliva as described in the following protocol.

Saliva Collection

Collect and preserve saliva as described by the Oragene™ device manufacturer. If the saliva sample is collected immediately prior to purification, incubate the sample at 50°C for 1 hour before starting the protocol. Otherwise, an overnight incubation at room temperature in the Oragene™ device is sufficient to release and preserve genomic DNA.

1. Set a water bath or heat block at 55°C.
2. Transfer 200 µL of saliva mixture from Oragene™ self-collection device into each well of a 96 Deep Well Plate and mix with 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal plate with a Foil Tape.
3. Incubate at 55°C for 10 minutes.
4. Add 200 µL 96–100% ethanol. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
5. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Purification Procedure Using 96-Well Plates

Introduction

The purification procedure is designed for purifying genomic DNA with 96-well plates with a vacuum manifold or centrifuge in a total time of **30–45 minutes**.

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Review **Elution Parameters** on page 13 to determine the suitable elution volume for your requirements
- Perform a 1 minute incubation step with PureLink® Genomic Elution Buffer
- Be sure to perform the recommended wash steps to obtain the best results
- If you are using water for elution, always use sterile water, pH 7.0–8.5
- Use the recommended vacuum pressure

Instrument Compatibility for 96-Well Plates

The PureLink® gDNA Binding Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink® Plates and be capable of collecting the filtrate (e.g., PureLink® Vacuum Manifold, page 43, UniVac™ 3 Vacuum Manifold System from Whatman, or QIAvac 96 from Qiagen).
- **Centrifuge:** Must be capable of centrifuging 96-well plates at ≥2250 × g, and accommodate a 5.75 cm microtiter plate stack.
- **Automated Liquid Handling Workstation:** The workstation must be equipped with a vacuum manifold and a vacuum source. The PureLink® gDNA Binding Plate is compatible for use on the Biomek® FX, Tecan Freedom EVO™, and BioRobot® Workstations.

  Note: If you are using the BioRobot® Workstation, you can perform elution using centrifugation or vacuum manifold as described on page 38.

Calibrating Vacuum for Use with 96-Well Plates

We recommend using a vacuum pressure of –6 to –12 inches Hg (–200 to –400 mbar or –150 to –300 mm Hg) to obtain the best results.

Using higher vacuum pressure than the recommended pressure may cause sample splattering or inefficient DNA binding, while using lower vacuum pressure will affect the elution resulting in lower recovery.

To check the vacuum pressure:

1. Place an unused PureLink® gDNA Binding Plate on top of the vacuum manifold and seal the plate with Foil Tape.
2. Apply vacuum and check the vacuum pressure on the vacuum regulator (usually attached to the manifold or a vacuum pump).
3. Adjust the vacuum pressure on the regulator to obtain the recommended pressure of –6 to –12 inches Hg.

   Note: During purification the vacuum pressure may exceed the recommended value.

Purification Procedure Using 96-Well Plates, Continued

Materials Needed

Components supplied by the user

- Lysates prepared as described on pages 27–33
- Vacuum manifold and a vacuum pump for 96-well plates (producing pressure of –6 to –12 inches Hg or –200 to –400 mbar or –150 to –300 mm Hg) or automated liquid handling workstations

  or
- Centrifuge with a swinging bucket rotor with 96-well plate carriers that have a plate height clearance of ~5.75 cm, and capable of centrifuging at ≥2250 × *g* at 25°C
- Optional: sterile, DNase-free 1.5 cm standard microtiter plates for elution
- Optional: sterile water, pH 7.0–8.5, if you are using water for elution

Components supplied with the Kit

- PureLink® Genomic Wash Buffers 1 and 2
- PureLink® Genomic Elution Buffer
- PureLink® gDNA Binding and Wash Plates
- 96 Deep Well Plates and Foil Tape

Note

- The purified gDNA can be eluted into 96 Deep Well Plates (supplied with the kit) or standard 1.5 cm microtiter plates (not supplied).
- The 96 Deep Well Plates can be reused. However, for elution, we recommend that you use a clean, **unused** 96 Deep Well Plate.
- If you are using automated liquid handling workstations for purification, you may need additional buffers depending on your type of workstation. Individual PureLink® Genomic Buffers are available, see page 43 for ordering information.

PureLink® Genomic DNA Wash Plate

The PureLink® Genomic DNA Wash Plate is designed to prevent cross contamination of the silica membrane when used with the PureLink® Vacuum Manifold or equivalent. The PureLink® Genomic DNA Wash Plate is a microtiter plate that is open on both sides allowing free flow of buffers.

The vacuum manifold is assembled with the wash plate placed underneath the binding plate. During washing, 96 separate channels are formed when the outlets on the binding plate protrude into the wash plate wells. This prevents any spraying of wash buffer onto the binding plate thereby reducing cross contamination and ethanol carry over. The wash plate is removed and discarded after the washing steps.

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.

Purification Using Centrifugation

1. Assemble the PureLink® gDNA Binding Plate onto a new or used 96 Deep Well Plate supplied with the kit. Ensure that the nozzles of the Binding Plate extend into the wells of the 96 Deep Well Plate.
2. Transfer each lysate (~640 µL) to a well of the PureLink® gDNA Binding Plate using a multichannel pipettor. Cover any unused wells with Foil Tape.
3. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes.
4. Discard the flow through and reassemble the PureLink® gDNA Binding Plate onto the used 96 Deep Well Plate.
5. Add 500 µL Wash Buffer 1 prepared with ethanol (page 35) into each well of the PureLink® gDNA Binding Plate.
6. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes.
7. Discard the flow through and reassemble the plate stack.
8. Add 500 µL Wash Buffer 2 prepared with ethanol (page 35) into each well of the PureLink® gDNA Binding Plate.
9. Centrifuge the stacked plates at ≥2250 × *g* for 15 minutes.

   Note: To ensure the complete drying of the membrane, do not seal the plate.
10. Discard the flow through and reassemble the PureLink® gDNA Binding Plate onto a **new** 96 Deep Well Plate supplied with the kit.
11. Add 50–200 µL PureLink® Genomic Elution Buffer to the center of the membrane in each well and incubate the plate for 1 minute at room temperature.

    Note: Review **Elution Parameters** on page 13 to choose a suitable elution volume for your needs.

    Centrifuge the stacked plates at ≥2250 × *g* for 3 minutes. *The purified gDNA is eluted in the Deep Well Plate.*
12. If desired, perform a second elution to increase recovery which lowers the overall concentration.
13. Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Purification Using Vacuum Manifold

1. Assemble the vacuum manifold as per the manufacturer's instructions.

   Brief instructions for assembling the PureLink® Vacuum Manifold are described below. Refer to the manual supplied with the manifold for details.

   a. Insert the waste tray into the manifold base.

   b. Insert one set of MTP/Multi96 plate adaptors into each side slot of the manifold base.

   c. Place the PureLink® Genomic DNA Wash Plate over adaptors to prevent any cross contamination. See page 35 for a description of the Wash Plate.

   d. Place the manifold lid on the manifold base to close the manifold.

   Note: The Wash Plate is not needed when using the UniVac™ 3 Vacuum Manifold (Whatman). To use the Wash Plate with QiaVac 96 Vacuum Manifold (Qiagen), you need the PureLink® Adaptor Frame (page 43).

2. Place the PureLink® gDNA Binding Plate onto the vacuum manifold designed to hold a 96-well plate.
3. Transfer the lysates (~640 µL) from each well of the Deep-Well Plate to a fresh well in the Binding Plate. Cover unused wells with Foil Tape.
4. Apply vacuum at room temperature until the lysate completely passes through the filter plate and release vacuum.
5. Add 1 mL Wash Buffer 1 prepared with ethanol (page 35) into each well of the Binding Plate.
6. Apply vacuum for 2 minutes at room temperature. Release vacuum.
7. Add 1 mL Wash Buffer 2 prepared with ethanol (page 35) into each well of the Binding Plate.
8. Apply vacuum for 2 minutes at room temperature. Release vacuum.
9. Disassemble the manifold to remove and discard the wash plate. Tap the Binding Plate on paper towels to remove any residual Wash Buffer from the nozzles. Reassemble the manifold with the binding plate.
10. Apply vacuum for 10 minutes at room temperature to allow membrane drying. Release vacuum.
11. Disassemble the manifold to remove the waste tray. Discard the waste tray contents.
12. Assemble the vacuum manifold with a Deep Well Plate or 1.5 cm microtiter plate for elution as per the manufacturer's instructions. See next page for brief instructions on assembling various vacuum manifolds with different types of adaptors and elution plates based on the type of plate used for elution.
13. Place the PureLink® gDNA Binding Plate onto the vacuum manifold.

Purification Using Vacuum Manifold, continued

14. Add 100–200 µL of PureLink® gDNA Elution Buffer to the center of the membrane in each well of the Binding Plate and incubate the plate for 1 minute at room temperature. **Note:** Review **Elution Parameters** (page 13) to choose a suitable elution volume.
15. Apply vacuum for 2 minutes at room temperature. Release vacuum. Disassemble the vacuum manifold to remove the elution plate. *The purified gDNA is eluted into the elution plate.*

Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Assembling Vacuum Manifold for Elution

The purified gDNA is eluted from the vacuum manifold in a 96 Deep Well Plate (supplied with the kit) or a standard 1.5-cm microtiter plate (not supplied). Based on your vacuum manifold, you may need to use adaptors with the elution plate to ensure the elution plate is aligned with Binding Plate.

PureLink® Vacuum Manifold (Life Technologies)

Brief instructions for assembling the PureLink® Vacuum Manifold for elution are described below. Refer to the manual supplied with the manifold for details.

For 96 Deep Well Plate

1. Insert one set of Round well adaptors followed by one set of Microtube Rack adaptors into each side slot of the manifold base.
2. Place the 96 Deep Well Plate supplied with the kit on the adaptors.
3. Place the manifold lid on the manifold base to close the manifold.

For 1.5-cm Microtiter Plate

1. Insert one set of MTP/Multi96 plate adaptors into each side slot of the manifold base.
2. Place the 1.5-cm microtiter plate (not supplied) on the adaptor.
3. Place the manifold lid on the manifold base to close the manifold.

   Note: Do not use >100 µL elution volume when using the microtiter plate for elution to prevent any contact of the Binding Plate nozzles with the eluate.

UniVac™ 3 Vacuum Manifold (Whatman)

Perform elution directly into the 96 Deep Well Plates supplied with the kit. There is no need for any adaptors. Elution into 1.5 cm microtiter plates or 1.5-mL racked microtubes is not recommended.

QiaVac 96 Vacuum Manifold (Qiagen)

- For elution into 96 Deep Well Plates supplied with the kit, use the manifold with a suitable adaptor of 20–22 mm height.
- For elution into 1.5-cm microtiter plates (not supplied), use the PureLink® Adaptor Frame from Life Technologies (page 43) with the manifold. Be sure to use vacuum pressure of up to –200 mbar to prevent any splashing of the eluate.
- For elution into 1.5-mL racked microtubes, there is no need for any adaptors.

Results and Troubleshooting

Analyzing DNA Yield and Quality

DNA Yield

After purification with PureLink® Genomic DNA Purification Kit, the yield of purified DNA can be estimated by UV absorbance at 260 nm or Quant-iT™ DNA Assay Kits.

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5–8.5.
2. Calculate the amount of DNA using formula:

DNA (µg) = A_{260} × 50 µg/(1 A_{260} × 1 mL) × dilution factor × total sample volume (mL)

For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (page 43) provide a rapid, sensitive, and specific method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

Each kit contains a state-of-the-art quantitation reagent and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers/fluorometers or the Qubit™ Fluorometer.

DNA Quality

Typically, DNA isolated using the PureLink® Genomic DNA Purification Kit has an A_{260}/A_{280} >1.80 when samples are diluted in Tris-HCl (pH 7.5) indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

DNA Length

Genomic DNA isolated with the PureLink® Genomic DNA Purification Kit is usually in the size range of 20–50 kb. To determine the exact size of DNA, perform Pulse-Field Gel Electrophoresis (PFGE) on an agarose gel.

The DNA isolated using the PureLink® Genomic DNA Kits is suitable for use in PFGE without ethanol precipitation or any additional steps. General guidelines for PFGE are described in this section. For details, refer to the manufacturer's recommendations.

For PFGE, load 20 µL (0.5–1 µg) purified DNA/lane in 10X BlueJuice™ Gel loading Buffer on a 1% agarose gel in 0.5X TBE using appropriate PFGE molecular weight DNA ladders. Perform electrophoresis at 6 V/cm for 15 hours at 14°C using a switch time of 1–7 seconds. The gel is stained with ethidium bromide after electrophoresis to visualize the DNA.

Expected Results

DNA Yield

The yield of genomic DNA obtained from various samples using the PureLink® Genomic DNA Mini Kits is listed below. The DNA quantitation was performed using UV absorbance at 260 nm. The yield is the total yield from 2 × 200 µL elutions.

Material	Amount	DNA Yield
E.coli cells	2×10^9	10–30 µg
HeLa cells	5×10^6	20–40 µg
293F cells	5×10^6	15–30 µg
Human Blood	200 µL	3–10 µg
Mouse Tail	1–1.2 cm	5–25 µg
Mouse Brain	25 mg	10–30 µg
Mouse Liver	25 mg	10–30 µg
Mouse Spleen	10 mg	10–40 µg

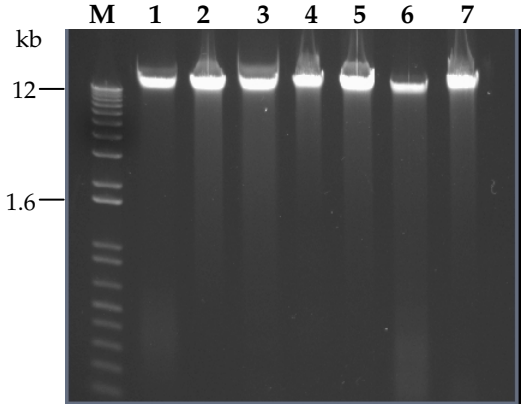
Note: The DNA yield varies with the sample and DNA content of the sample.

DNA Quality

Genomic DNA isolated from various samples was analyzed by agarose gel electrophoresis on a 1% E-Gel® agarose gel.

Samples on the gel are:

Lane M: 1 Kb Plus DNA Ladder
Lane 1: 200 ng DNA isolated from Gram positive bacteria (2×10^9 cells)
Lane 2: 200 ng DNA isolated from Gram negative bacteria, *E. coli* (2×10^9 cells)
Lane 3: 200 ng DNA isolated from human 293F (5×10^6 cells)
Lane 4: 200 ng DNA isolated from human whole blood (200 µL)
Lane 5: 200 ng DNA isolated from rat brain tissue (20 mg)
Lane 6: 200 ng DNA isolated from human saliva (200 µL of Oragene™ sample)
Lane 7: 200 ng DNA isolated from rat liver tissue (20 mg)



Troubleshooting

Introduction

Refer to the following table to troubleshoot any problems you may encounter with the PureLink® Genomic DNA Kits.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add Proteinase K during lysis. • For tissues, cut the tissue into smaller pieces and ensure the tissue is completely immersed in the Digestion Buffer to obtain optimal lysis. • If incomplete lysis is observed, increase the digestion time or amount of Proteinase K used for lysis.
	Poor quality of starting material	Be sure to use fresh sample and process immediately after collection or freeze the sample at –80°C or in liquid nitrogen. The yield and quality of DNA isolated is dependent on the type and age of the starting material.
	PureLink® Spin Column or Binding Plate is clogged	Make sure that the lysate is clear when the lysate is loaded on to the spin column or plate. Remove any particulate or viscous material by centrifugation prior to loading the lysate on to the spin column or plate.
	Incorrect binding conditions	• Be sure to add PureLink® Lysis/Binding Buffer and 96–100% ethanol to the lysate prior to loading the samples on the spin column or Binding Plate. Mix the sample properly with Binding Buffer and ethanol by vortexing. • Avoid overloading the column or plate.
	Ethanol not added to Wash Buffers 1 and 2	Be sure to add 96–100% ethanol to Wash Buffers 1 and 2 as indicated on the label.
	Incorrect elution conditions	• Add elution buffer and perform incubation for 1 minute with elution buffer before centrifugation. • To recover more DNA, perform a second elution step.
	DNA is sheared or degraded	• Avoid repeated freezing and thawing of samples to prevent any DNA damage. • Maintain a sterile environment while working to avoid any contamination from DNases.

Problem	Cause	Solution
Dark colored eluate or discolored membrane (mammalian tissue, mouse tails, or blood samples only)	Pigments from tissues or heme from blood bind to the silica matrix and co-elute with DNA	• Be sure to add ethanol to the lysate prior to loading the lysate on to the spin column or plate. The ethanol prevents the pigments from sticking on the silica matrix. • Perform centrifugation of the lysate at a higher speed and longer time prior to loading the lysate on to the column or plate. • If the problem persists, perform an additional wash step with 500 µL Wash Buffer 1 to obtain a total of two 500 µL wash steps with Wash Buffer 1 followed by a single 500 µL wash with Wash Buffer 2.
RNA contamination	Silica membrane binds total nucleic acid present in the sample	Perform RNase digestion step during sample preparation.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Traces of ethanol from the Wash Buffer 2 can inhibit downstream enzymatic reactions. • To remove Wash Buffer 2 from spin columns, discard Wash Buffer 2 flow through. Place the spin column into the Wash Tube and centrifuge the spin column at maximum speed for 2–3 minutes to completely dry the column. • To remove any traces of Wash Buffer 2 from the Binding Plate and dry the membrane, centrifuge the plate stack at ≥2250 × *g* for 15 minutes or apply vacuum for 10 minutes. The plate can also be warmed at 70ºC for 10 minutes to evaporate any ethanol.
	Presence of salt in purified DNA	• Use the correct order of Wash Buffers for washing. Always wash with Wash Buffer 1 followed by washing with Wash Buffer 2. • Always maintain a ratio of 1:1:1 for Sample:Binding Buffer:Ethanol.
Low elution volume or sample cross-contamination	Incorrect vacuum pressure	• Make sure the vacuum manifold is sealed tightly and there is no leakage. A vacuum pressure of –6 to –12 inches Hg (–200 to –400 mbar or –150 to –300 mm Hg) is required for best results. • To avoid any cross contamination and ensure proper contact between the PureLink® Genomic Binding Plate and elution plate, raise the elution plate in the vacuum manifold using adaptors as described on page 38.

Appendix

Safety

WARNING! GENERAL CHEMICAL HANDLING. For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

The PureLink® Genomic Lysis/Binding Buffer and Wash Buffer 1 contain guanidine hydrochloride. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/%2029cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**.

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at:
www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Accessory Products

Additional Products

The following products are also available from Life Technologies. For more details on these products, visit **www.lifetechnologies.com** or contact Technical Support (page 45).

Product	Quantity	Catalog No.
PureLink® Genomic DNA Mini Kit	10 preps	K1820-00
PureLink® Genomic Digestion Buffer	70 mL	K1823-01
PureLink® Genomic Lysis/Binding Buffer	80 mL	K1823-02
PureLink® Genomic Wash Buffer 1	100 mL	K1823-03
PureLink® Genomic Wash Buffer 2	75 mL	K1823-04
PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	160 mL	K1823-05
Proteinase K (20 mg/mL)	5 mL	25530-049
Foil Tape	50 pieces	12261-012
PureLink® Vacuum Manifold	1 each	K2110-01
PureLink® Vacuum Regulator	1 each	K2110-02
PureLink® Adaptor Frame	1 each	K2110-03
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Phosphate Buffered Saline (PBS), 1X	500 mL	10010-023

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats for your convenience.

A large variety of DNA ladders is available from Life Technologies for sizing DNA.

For more details on these products, visit **www.lifetechnologies.com** or contact Technical Support (page 45).

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

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