invitrogen™

PureLink™ Pro 96 Genomic DNA Kit

For purification of genomic DNA

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User Manual

Contents

Experienced Users Procedure

Introduction

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

Step	Action		
Preparing lysates	Prepare the lysate using an appropriate sample preparation protocol as follows:		
	Sample	**Amount**	**Page**
	Mammalian cells, tissues and mouse tail	5×10^6 cells, ≤25 mg tissue, 0.5–1 cm tail	11, 12
	Blood	≤200 µl nonnucleated blood 5–10 µl nucleated blood	11
	Bacteria	Up to 2×10^9 cells	14
	Yeast cells	Up to 5×10^7 cells	16
	FFPE tissue	1–8 sections (5–15 µm thick; ~20–50 mm^2 in area)	17
	Buccal Swab	Human buccal swabs	18
	Oragene™ preserved Saliva	Up to 200 µl	19

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Experienced Users Procedure, Continued

Step	Action
Purification Procedure with Centrifugation	Perform all centrifugation steps at 25°C. 1. Assemble the PureLink™ Pro 96 gDNA Filter Plate onto a new or used 96 Deep Well Block. 2. Transfer each lysate (~640 µl) to a well of the gDNA Filter Plate using a multichannel pipettor. Unused wells should be covered with Foil Tape. 3. Centrifuge the stacked plates at ≥2,100 × g for 5–10 minutes. 4. Discard flow through and reassemble the gDNA Filter Plate onto the 96 Deep Well Block. 5. Add 500 µl Wash Buffer 1 prepared with ethanol (page 8) into each well of the gDNA Filter Plate. 6. Centrifuge the stacked plates at ≥2,100 × g for 2–5 minutes. 7. Discard the flow through and reassemble the plate stack. 8. Add 500 µl Wash Buffer 2 prepared with ethanol (page 8) into each well of the gDNA Filter Plate. 9. Centrifuge stacked plates at ≥2,100 × g for 10 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 gDNA Filter Plate onto a **new** 96 Deep Well Block. 11. Add 50–200 µl PureLink™ Pro 96 Genomic Elution Buffer to the center of the membrane in each well and incubate the plate for 1 minute at room temperature. 12. Centrifuge the stacked plates at ≥2,100 × g for 3 minutes. *The purified gDNA is eluted in the Deep Well Block.* 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 Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Continued on next page

Experienced Users Procedure, Continued

Step	Action
Purification Procedure with Vacuum	1. Assemble the vacuum manifold as per the manufacturer's instructions. 2. Place the PureLink™ gDNA Filter Plate onto the vacuum manifold. 3. Transfer the lysates (~640 µl) from each well of the Deep-Well Block to a fresh well in the gDNA Filter Plate. Unused wells should be covered with Foil Tape. 4. Apply vacuum for 1–2 minutes until the lysate passes through the gDNA Filter Plate. Release the vacuum. 5. Add 1 ml Wash Buffer 1 prepared with ethanol (page 8) into each well of the gDNA Filter Plate. 6. Apply vacuum for 2 minutes. Release the vacuum. 7. Add 1 ml Wash Buffer 2 prepared with ethanol (page 8) into each well of the gDNA Filter Plate. 8. Apply vacuum for 2 minutes. Release the vacuum. 9. Blot the gDNA Filter Plate on paper towels to remove any residual liquid from the nozzles. Replace the gDNA Filter Plate on the manifold. 10. Apply vacuum for 10 minutes to dry the membrane. Release the 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. Place the gDNA Filter Plate onto the vacuum manifold. 13. Add 100–200 µl of PureLink™ Pro 96 Genomic Elution Buffer to the center of the membrane and incubate the plate for 1 minute at room temperature. 14. Apply vacuum for 2 minutes. Release the vacuum. Disassemble the vacuum manifold to remove the elution plate. *The purified gDNA is eluted into the elution plate.* 15. Use the purified gDNA for the desired downstream application. Otherwise, cover the wells with Foil Tape, and store at 4ºC (short-term) or –20°C (long-term).

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Kit Contents and Storage

Shipping and Storage

All components of the PureLink™ Pro 96 Genomic DNA Kit are shipped at room temperature.

Upon receipt, store all components at room temperature.

Note: The PureLink™ Pro 96 Proteinase K 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 and RNase A at 4ºC.

PureLink™ Pro 96 Genomic DNA Kit Contents

The components included in the PureLink™ Pro 96 Genomic DNA Kit are listed below.

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

Box Contents	Quantity
PureLink™ Pro 96 Genomic Lysis/Binding Buffer	80 ml
PureLink™ Pro 96 Genomic Digestion Buffer	70 ml
PureLink™ Pro 96 Genomic Wash Buffer 1	2 × 100 ml
PureLink™ Pro 96 Genomic Wash Buffer 2	2 × 75 ml
PureLink™ Pro 96 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
PureLink™ Pro 96 Proteinase K (20 mg/ml) in storage buffer (proprietary)	8 ml
PureLink™ Genomic DNA Filter Plate	4 plates
96 Deep Well Blocks	2 × 6 plates
PureLink™ Foil Tape	20/pack

Introduction

About the Kit

Kit Usage

The PureLink™ Pro 96 Genomic DNA Kit allows 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 by 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™ Pro 96 Genomic DNA 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. 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™ Pro 96 Genomic Binding Buffer that allows genomic DNA to bind the PureLink™ gDNA Filter Plate. The DNA binds to the membrane in plate and impurities are removed by thorough washing with Wash Buffers. The genomic DNA is then eluted in low salt Elution Buffer.

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About the Kit, Continued

Advantages

- 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 a 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

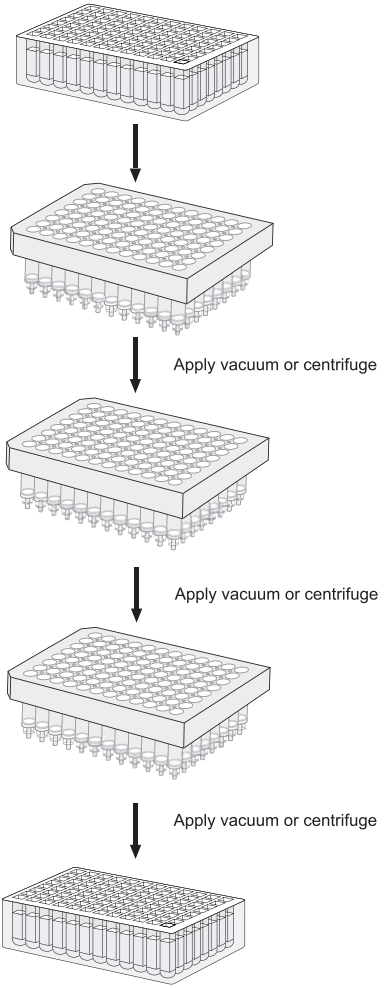
Kit Specifications

Plate Dimensions:	Standard SBS (Society for Biomolecular Screening) footprint
Starting Material:	Varies (see page 9)
Binding Capacity/Plate:	~0.5 mg nucleic acid
gDNA Filter Plate Capacity:	1 ml
Deep-Well Block Capacity:	1.0 ml (0.75 ml without contacting nozzles)
Centrifuge Compatibility:	Capable of centrifuging at ≥2,250 × g Bucket depth 7 cm
Elution Volume:	50–200 µl
DNA Yield:	Varies
DNA Size:	20–50 kb

Experimental Overview

Workflow

The flow chart for purifying genomic DNA using the PureLink™ Pro 96 Genomic DNA Kit is shown below.



Methods

General Guidelines

- The PureLink™ Pro 96 Genomic 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.
- 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). Since safety requirements for use and handling of blood and tissue samples may vary at individual institutions, consult the health and safety guidelines and/or officers at your institution.
- When processing blood and tissue samples, the supernatants collected during wash steps contain biohazardous waste. Dispose of the supernatants and collection tubes/plates appropriately as biohazardous waste.

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

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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 table below.

The PureLink™ Pro 96 Genomic DNA Kit is 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	Up to 25 mg (up to 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)	Up to 200 µl (single pass)
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*)	Up to 2×10^9 cells
Gram positive bacteria (*e.g.*, Bacillus)	Up to 2×10^9 cells
Yeast cells	Up to 5×10^7 cells
FFPE tissue	1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2.
Oragene™ preserved saliva	Up to 200 µl

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General Guidelines, Continued

Whole Blood Samples

The PureLink™ Pro 96 Genomic DNA Kit is 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.

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 PureLink™ Pro 96 Proteinase K is used for efficient lysis of tissues/cells. Proteinase K digestion is performed using an optimized buffer formulation, PureLink™ Pro 96 Genomic Digestion Buffer, for optimal enzymatic activity.

Paraffin Extraction

Prior to the isolation of gDNA from FFPE (formalin-fixed, paraffin-embedded) tissue samples, the paraffin must be removed.

If using xylene for paraffin extraction, use the appropriate precautions for handling xylene and dispose of xylene in compliance with established institutional guidelines.

CitriSolv™ Clearing Agent is recommended as a biodegradable alternative to xylene.

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Processing Fewer than 96 Samples

You can use a portion of the PureLink™ Pro 96 gDNA Filter Plate to isolate genomic DNA from fewer than 96 samples. **Each well in the plates can only be used once**.

To process fewer than 96 samples:

1. Cover the entire surface of the gDNA Filter Plate with Foil Tape (see page 30).
2. Just prior to binding the DNA to the gDNA Filter Plate, score the foil around the wells to be used with a clean, sharp blade. Peel away the foil to expose the wells containing the samples.
3. Keep all unused wells in the plate sealed with Foil Tape during purification to obtain a uniform vacuum and avoid contaminating unused wells.

Important: When performing **Purification by Vacuum Manifold**, only the plate that is inserted into the vacuum manifold lid needs to be covered. **Do not** cover the bottom plate in the manifold base, or the nozzles of the top plate will not fit into the wells of the bottom plate, preventing a tight vacuum seal.

Instrument Compatibility

The PureLink™ gDNA Filter Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink™ 96 Well Plates (half-skirted filter plate) and be capable of collecting the filtrate (e.g. EveryPrep™ Universal Vacuum Manifold from Invitrogen).
- **Centrifuge:** The centrifuge must be capable of centrifuging 96-well plates at ≥2,100 × g, and accommodate a 7.0 cm microtiter plate stack.
- **Automated Liquid Handling Workstation:** The workstation must be equipped with a vacuum manifold and a vacuum source, and accommodate the PureLink™ 96 Well Plate (half-skirted filter plate).

Preparing Buffer Solutions

Introduction

Prepare the required buffer solutions **before** beginning the purification.

PureLink™ Pro 96 Genomic Wash Buffer 1

Add 150 ml of 96–100% ethanol to PureLink™ Pro 96 Genomic Wash Buffer 1, and mix well. Mark the label to indicate that ethanol is added. Store wash buffer with ethanol at room temperature.

PureLink™ Pro 96 Genomic Wash Buffer 2

Add 175 ml 96–100% ethanol to PureLink™ Pro 96 Genomic Wash Buffer 2, and mix well. Mark the label to indicate that ethanol is added. Store wash buffer with ethanol at room temperature.

PureLink™ Pro 96 Genomic Digestion Buffer

When preparing lysates from **mammalian tissue or mouse/rat tail** (page 12), **blood spots** (page 13), **gram negative bacteria** (page 14), or **FFPE tissue** (page 17), prepare a master mix of PureLink™ Pro 96 Genomic Digestion Buffer with PureLink™ Pro 96 Proteinase K as follows:

Reagent	Volume/Sample
PureLink™ Pro 96 Genomic Digestion Buffer	180 µl
PureLink™ Pro 96 Proteinase K	20 µl

Use 200 µl of PureLink™ Pro 96 Genomic Digestion Buffer with Proteinase K for each well.

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Preparing Buffer Solutions, Continued

PureLink™ Pro 96 Genomic Lysis/Binding Buffer

When preparing lysates from **mammalian tissue or mouse/rat tail** (page 12), **blood spots** (page 13), **gram negative bacteria** (page 14), **yeast** (page 16), or **FFPE tissue** (page 17), prepare a master mix of PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol as follows:

Reagent	Volume/Sample
PureLink™ Pro 96 Genomic Lysis/Binding Buffer	200 µl
96–100% Ethanol	200 µl

Use 400 µl of PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol for each well.

Lysozyme Digestion Buffer

When preparing lysates from gram positive bacteria (page 15), prepare **fresh** Lysozyme Digestion Buffer with the following composition:

Reagent	Amount
Tris-HCl, pH 8.0	25 mM
EDTA	2.5 mM
Triton® X-100	1%
Lysozyme	20 mg/ml

Use 180 µl of Lysozyme Digestion Buffer for each well.

Zymolase Buffer

When preparing lysates from yeast (page 16), prepare **fresh** Zymolase Buffer with the following composition:

Reagent	Amount
Sorbitol	1 M
EDTA (sodium salt)	10 mM
β-mercaptoethanol	14 mM

Use 500 µl of Zymolase Buffer for each well.

Preparing Lysates

Materials Needed

- 96–100% ethanol
- Sample for DNA isolation (see page 5 for recommended starting amount)
- Water baths or heat blocks
- PureLink™ Pro 96 Genomic Lysis/Binding Buffer
- Foil Tape
- 96 Deep Well Blocks
- PureLink™ Pro 96 Proteinase K
- PureLink™ Pro 96 Genomic Digestion Buffer
- RNase A (supplied with kit)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate (page 30)
- Lysozyme Buffer for gram positive bacteria (see page 9)
- Zymolase Buffer and Zymolase for yeast (see page 9)
- CitriSolv™ Clearing Agent (Fisher catalog. 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

Follow the recommendations below for the best results:

- 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

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 30 for ordering information.

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Preparing Lysates, Continued

Mammalian Cells and Blood Lysate

1. Set a water bath or heat block at 55°C.
2. Add 20 µl PureLink™ Pro 96 Proteinase K to each well of a 96 deep Well Block.
3. Process cells or blood samples as follows:
   - **Adherent cells (≤5 × 10^6 cells):** Remove growth medium from the culture plate and harvest cells by trypisinization or a method of choice. Resuspend the cells in 200 µl PBS.
   - **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.
   - **Blood samples:** Add ≤200 µl fresh or frozen blood sample to a sterile microtiter plate. Adjust the final sample volume to 200 µl with PBS.
4. Transfer 200 µl of sample in PBS to each well of a 96 Deep Well Block containing Proteinase K (from Step 2).
5. Add 20 µl RNase A (supplied in the kit) to each well. Seal the plate with Foil Tape. Mix well by brief vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
6. Incubate at room temperature for 2 minutes.
7. Remove Foil Tape and add 200 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer. 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. Incubate at 55°C for 10 minutes to promote protein digestion.
9. Remove Foil Tape and add 200 µl 96–100% ethanol to the lysate. 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.
10. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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Mammalian Tissue and Mouse/Rat Tail Lysate

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 Block:
   - Up to 25 mg of minced mammalian tissue
   - 1 cm mouse or 0.5 cm rat tail clip

   Note: For mammalian tissues that produce a viscous lysate (e.g., spleen tissue), start with 10 mg of minced tissue.
3. Add 200 µl PureLink™ Genomic Pro 96 Digestion Buffer with Proteinase K (see page 9) to each well of the 96 Deep Well Block. Ensure the tissue is completely immersed in the buffer. Seal the plate with Foil Tape and mix well by vortexing.
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 >2,100 × g for 3 minutes at room temperature to remove any particulate materials.
6. Remove Foil Tape and add 20 µl RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at room temperature for 2 minutes.
8. Remove Foil Tape and add 400 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol (see page 9). 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.
9. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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Blood Spots

1. Set a water bath or heat block at 55°C.
2. Place 2–5 punches from a dried blood spot (2–3 mm in size) in a well of a 96 Deep Well Block.
3. Add 200 µl PureLink™ Pro 96 Genomic Digestion Buffer with Proteinase K (see page 9) to each well. The pieces must be completely immersed in buffer. Seal the plate with Foil Tape and mix well by vortexing.
4. Incubate at 55°C with occasional vortexing for 30 minutes.
5. Centrifuge the sample at >2,100 × g for 2–3 minutes at room temperature to pellet paper fibers. Transfer samples to a clean, 96 Deep Well Block.
6. Remove Foil Tape and add 20 µl RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at room temperature for 2 minutes.
8. Remove Foil Tape and add 400 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol (see page 9). 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.
9. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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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 bacteria (~1 ml of overnight *E. coli* culture) in a 96 Deep Well Block by centrifugation at >2,100 × g for 10 minutes.
3. Resuspend the cell pellet in 200 µl PureLink™ Pro 96 Genomic Digestion Buffer with Proteinase K (see page 9) to lyse the cells. Seal the plate with Foil Tape and mix well by vortexing.
4. Incubate the tube at 55°C with occasional vortexing until lysis is complete (30 minutes to 4 hours).
5. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
6. Remove Foil Tape and add 20 µl RNase A (supplied in the kit) 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.
7. Incubate at room temperature for 2 minutes.
8. Remove Foil Tape and add 400 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol (see page 9). 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.
9. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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Gram Positive Bacterial Cell Lysate

1. Set two water baths or heat blocks at 37ºC and 55°C, respectively.
2. Prepare **fresh** Lysozyme Digestion Buffer (see page 9). You will need 180 µl buffer per sample.
3. Harvest up to 2×10^9 Gram positive cells in a 96 Deep Well Block by centrifugation at 2,100 × g for 10 minutes.
4. Resuspend the cell pellet in 180 µl Lysozyme Digestion Buffer with Lysozyme (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. Remove Foil Tape and add 20 µl PureLink™ Pro 96 Proteinase K.
7. Add 200 µl PureLink™ Pro 96 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.
8. Incubate at 55°C for 30 minutes.
9. Remove Foil Tape and add 200 µl 96–100% ethanol to the lysate. 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.
10. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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Yeast Cells Lysate

1. Set 2 water baths or heat blocks at 37°C and 55°C, respectively.
2. Prepare **fresh** Zymolase Buffer (see page 9). You will need 500 µl buffer per sample.
3. Harvest up to 5×10^7 yeast cells by centrifugation in a 96 Deep Well Block.
4. Resuspend the cell pellet in 500 µl Zymolase Buffer (Step 2). Add 15 units of Zymolase (lyticase) enzyme and incubate at 37°C for 1 hour to generate spheroplasts.
5. Centrifuge at 3,000 × g for 10 minutes at room temperature to pellet the spheroplasts.
6. Resuspend the spheroplasts in 180 µl PureLink™ Pro 96 Genomic Digestion Buffer.
7. Remove Foil Tape and add 20 µl PureLink™ Pro 96 Proteinase K. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
8. Incubate at 55°C for 45 minutes.
9. Remove Foil Tape and add 20 µl RNase A (supplied in the kit) to the lysate. Seal the plate with Foil Tape, and mix well by vortexing.
10. Incubate at room temperature for 2 minutes.
11. Remove Foil Tape and add 400 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol (see page 9). 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.
12. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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FFPE Tissue Lysate

1. Set 2 water baths or heat blocks at 37ºC and 50°C, respectively.
2. Place 1–8 sections of tissue (5–15 µm thickness; 20–50 mm^2 surface area), of no more than 20 mg into each well of a 96 Deep Well Block.
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.
4. Centrifuge at >2,100 × g 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 >2,100 × g 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 200 µl PureLink™ Pro 96 Genomic Digestion Buffer with Proteinase K (see page 9). Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
10. Incubate at 50°C until adequate digestion is achieved (3 hours to overnight).
11. Centrifuge the lysate at >2,100 × g for 3 minutes at room temperature to remove any particulate materials.
12. Remove Foil Tape and add 20 µl RNase A (supplied in the kit) to the lysate. Seal the plate with Foil Tape, and mix well by vortexing.
13. Incubate at room temperature for 2 minutes.
14. Remove Foil Tape and add 400 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer with ethanol (see page 9). 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.
15. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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Human Buccal Swab Lysate

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 PureLink™ Pro 96 Proteinase K into the wells of a 96 Deep Well Block.
4. Transfer 200 µl swab lysate to 96 Deep Well Block containing Proteinase K (Step 3).
5. Add 200 µl PureLink™ Pro 96 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. Remove Foil Tape and 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.
8. Proceed immediately to purification protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

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Oragene™ Preserved Saliva

Process up to 200 µl Oragene™ preserved saliva as described below.

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 Block and mix with 200 µl PureLink™ Pro 96 Genomic Lysis/Binding Buffer. Seal plate with Foil Tape.
3. Incubate at 55°C for 10 minutes.
4. Remove Foil Tape and 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 protocol for **Centrifugation** (page 21), **Vacuum Manifold** (page 24), or **EveryPrep™ Universal Manifold** (page 31).

Purification Using Centrifugation

Introduction

The purification procedure is designed for purifying genomic DNA with 96-well plates with a centrifuge in a total time of **30–45 minutes**. All steps are performed at room temperature.

Materials Needed

- Lysates prepared as described on pages 11-19
- Centrifuge capable of reaching ≥2,100 × g with buckets that can accommodate 96-well plates with a plate height clearance of 7.0 cm.
- *Optional*: sterile water, pH >7.0, if you are using water for elution
- PureLink™ Pro 96 Genomic Wash Buffers 1 and 2 with ethanol
- PureLink™ Pro 96 Genomic Elution Buffer
- PureLink™ gDNA Filter Plate
- 96 Deep Well Blocks and Foil Tape

Note

- The purified gDNA can be eluted into 96 Deep Well Blocks (supplied with the kit) or standard 1.5 cm microtiter plates (not supplied).
- The 96 Deep Well Blocks can be reused. However, for elution, we recommend that you use a clean, **unused** 96 Deep Well Block.

Continued on next page

Purification Using Centrifugation, Continued

Purification Using Centrifugation

1. Place the PureLink™ gDNA Filter Plate onto a 96 Deep Well Block (supplied with the kit). Ensure that the nozzles of the gDNA Filter Plate extend into the wells of the 96 Deep Well Block.
2. Transfer each lysate (~640 µl) to a well of the gDNA Filter Plate using a multichannel pipettor. Unused wells should be covered with Foil Tape.
3. Centrifuge the stacked plates at ≥2,100 × g for 5–10 minutes.
4. Discard the flow through and reassemble the gDNA Filter Plate onto the used 96 Deep Well Block.
5. Add 500 µl Wash Buffer 1 prepared with ethanol (page 8) into each well of the PureLink™ gDNA Filter Plate.
6. Centrifuge the stacked plates at ≥2,100 × g for 2–5 minutes.
7. Discard the flow through and reassemble the plate stack.
8. Add 500 µl Wash Buffer 2 prepared with ethanol (page 8) into each well of the gDNA Filter Plate.
9. Centrifuge the stacked plates at ≥2,100 × 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 gDNA Filter Plate onto a **new** 96 Deep Well Block supplied with the kit.
11. Add 50–200 µl PureLink™ Pro 96 Genomic Elution Buffer to the center of the membrane in each well and incubate the plate for 1 minute at room temperature.
12. Centrifuge the stacked plates at ≥2,100 × g for 3 minutes.

    The purified gDNA is eluted in the Deep Well Block.
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. Otherwise, cover the wells with Foil Tape, and store at 4ºC (short-term), or –20°C (long-term).

Continued on next page

Purification Using a Vacuum Manifold

Introduction

The purification procedure is designed for purifying genomic DNA with 96-well plates with a vacuum manifold in a total time of **30–45 minutes**. All steps are performed at room temperature. For a protocol using the EveryPrep™ Universal Vacuum Manifold, see page 31.

Materials Needed

- Lysates prepared as described on pages 11-19
- Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg) or automated liquid handling workstations
- *Optional*: Centrifuge capable of reaching ≥2,100 × g with buckets that can accommodate 96-well plates with a plate height clearance of 7.0 cm.
- *Optional*: sterile water, pH >7.0, if you are using water for elution
- PureLink™ Pro 96 Genomic Wash Buffers 1 and 2 with ethanol
- PureLink™ Pro 96 Genomic Elution Buffer
- PureLink™ gDNA Filter Plate
- 96 Deep Well Blocks and Foil Tape

Note

- Elute purified gDNA into 96 Deep Well Blocks (supplied with the kit) or standard 1.5 cm microtiter plates (not supplied).
- The 96 Deep Well Blocks can be reused. However, for elution, we recommend that you use a clean, **unused** 96 Deep Well Block.
- If you are using automated liquid handling workstations for purification, you may need additional buffers depending on your type of workstation. Individual PureLink™ Pro 96 Genomic Buffers are available, see page 30 for ordering information.

Continued on next page

Purification Using a Vacuum Manifold,
Continued

Calibrating Vacuum for Use with 96-Well Plates

Use a vacuum pressure of –12 to –15 in. Hg, or reduce the vacuum pressure until a flow rate of 1–2 drops per second is achieved to obtain the best results.

Using higher vacuum pressure than recommended 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™ 96 gDNA Filter Plate on top of the vacuum manifold. 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 –12 to –15 in. Hg.

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

Continued on next page

Purification Using a Vacuum Manifold,
Continued

Purification Using Vacuum Manifold

1. Set up the vacuum manifold using manufacturer's recommendations.
   If you are using an automated liquid handling workstation, prepare the workstation deck as recommended by the manufacturer.
2. Place the PureLink™ gDNA Filter Plate on top of the manifold.
3. Transfer the lysates (~640 µl) from each well of the Deep-Well Block to a fresh well in the gDNA Filter Plate. Unused wells should be covered with Foil Tape.
4. Apply vacuum for 1–2 minutes until the lysate passes through the gDNA Filter Plate. Release the vacuum.
5. Add 1 ml Wash Buffer 1 prepared with ethanol (page 8) into each well of the gDNA Filter Plate.
6. Apply vacuum for 2 minutes. Release the vacuum.
7. Add 1 ml Wash Buffer 2 prepared with ethanol (page 8) into each well of the gDNA Filter Plate.
8. Apply vacuum for 2 minutes. Release the vacuum.
9. Place the gDNA Filter Plate on a stack of paper towels, and pat firmly to remove any residual liquid from the nozzles. Replace the gDNA Filter Plate on the manifold.
10. Apply vacuum for 10 minutes to allow membrane drying. Release the vacuum.
11. Disassemble the manifold to remove the waste tray. Discard the waste tray contents.
12. Assemble the vacuum manifold with a 96 Deep Well Block or 1.5 cm microtiter plate in the vacuum manifold.
13. Place the PureLink™ gDNA Filter Plate onto the vacuum manifold.
14. Add 100–200 µl of PureLink™ gDNA Elution Buffer to the center of the membrane in each well of the gDNA Filter Plate and incubate the plate for 1 minute.
15. Apply vacuum for 2 minutes. Release the 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. Otherwise, cover the wells with Foil Tape, and store at 4ºC (short-term) or –20°C (long-term).

Analyzing DNA Yield

Estimating DNA Yield

After purification with PureLink™ Pro 96 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 30) 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.

Analyzing DNA Quality

Estimating DNA Quality

Typically, DNA isolated using the PureLink™ Pro 96 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.

Estimating DNA Length

Genomic DNA isolated with the PureLink™ Pro 96 Genomic DNA Purification Kit is usually in the size range of 20–50 kb. To determine the exact size of DNA, perform PFGE (Pulse-Field Gel Electrophoresis) on an agarose gel.

The DNA isolated using the PureLink™ Pro 96 Genomic DNA Kit is suitable for use in PFGE without ethanol precipitation or any additional steps. General guidelines for PFGE are described below. 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 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)

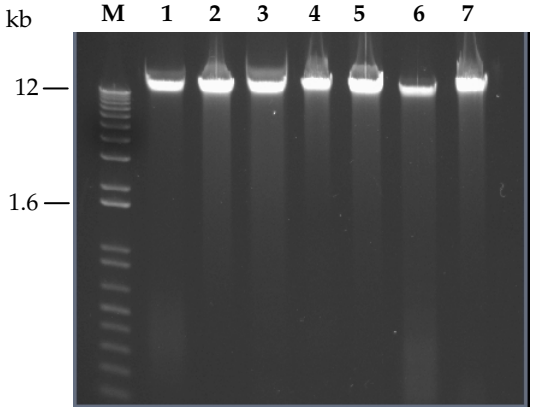
Lane 3: 200 ng DNA isolated from human 293F (5×10^6 cells)

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 table below to troubleshoot any problems you may encounter with the PureLink™ Pro 96 Genomic DNA Kit. For additional assistance, contact **Technical Support** (see page 31).

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™ Pro 96 gDNA Filter Plate is clogged	Make sure that the lysate is clear when the lysate is loaded on to the plate. Remove any particulate or viscous material by centrifugation prior to loading the lysate on to the 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 gDNA Filter Plate. Mix the sample properly with buffer and ethanol by vortexing. • Avoid overloading the 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 work environment to avoid contamination from DNases.

Continued on next page

Troubleshooting, Continued

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 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 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 A 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 any traces of Wash Buffer 2 from the gDNA Filter Plate and dry the membrane, centrifuge the plate stack at >2,100 × 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 –12 to –15 in. Hg is required to obtain the best results. • To avoid any cross contamination ensure proper contact between the PureLink™ gDNA Filter Plate and elution plate.

Appendix

Accessory Products

Additional Products

The following products are also available from Invitrogen. For more details on these products, visit www.invitrogen.com or contact Technical Support (page 33).

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
EveryPrep™ Universal Vacuum Manifold	1 manifold	K2111-01
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 in different agarose percentages and well formats, designed for fast, convenient electrophoresis of DNA samples.

A large variety of DNA ladders are available from Invitrogen for sizing DNA.

For more details on these products, visit our website at www.invitrogen.com or contact **Technical Support** (see page 31).

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold

Introduction

Instructions are provided below to purify DNA using the EveryPrep™ Universal Vacuum Manifold (page 30). Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for detailed instructions on operation with the 96 Well Top Plate. All steps are performed at room temperature.

Materials Needed

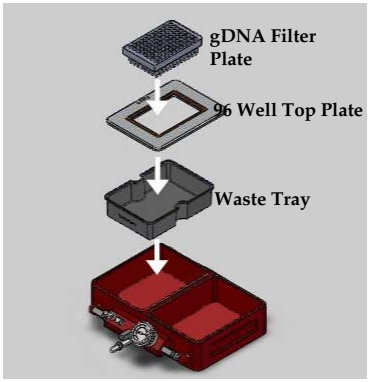
- Lysates prepared as described on pages 11-19
- Vacuum pump (producing pressure of 12–15 in. Hg)
- *Optional*: sterile, DNase-free 1.5 cm standard microtiter plates for elution
- *Optional*: sterile water, pH >7.0, if you are using water for elution
- PureLink™ Pro 96 Genomic Wash Buffers 1 and 2
- PureLink™ Pro 96 Genomic Elution Buffer
- PureLink™ gDNA Filter Plate
- 96 Deep Well Blocks and Foil Tape

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

EveryPrep™ Universal Vacuum Manifold Assembly

1. Place the Waste Tray in the Binding Chamber, cover the top with the 96 Well Top Plate, and place the PureLink™ gDNA Filter Plate over the Top Plate.



2. Proceed to **Binding DNA**, below.

Binding DNA

1. Transfer the lysates (~640 µl) from each well of the Deep-Well Block to a fresh well in the gDNA Filter Plate. Unused wells should be covered with Foil Tape.
2. Apply vacuum for 1–2 minutes until the lysate passes through the gDNA Filter Plate. Release the vacuum.
3. Proceed to **Washing DNA**, below.

Washing DNA

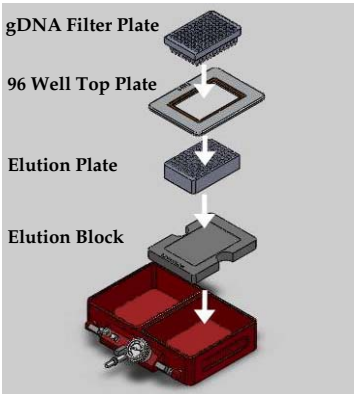
1. Add 1 ml Wash Buffer 1 prepared with ethanol (page 8) into each well of the gDNA Filter Plate.
2. Apply vacuum for 2 minutes. Release the vacuum.
3. Add 1 ml Wash Buffer 2 prepared with ethanol (page 8) into each well of the gDNA Filter Plate.
4. Apply vacuum for 2 minutes. Release the vacuum.
5. Place the gDNA Filter Plate on a stack of paper towels, and pat firmly to remove any residual liquid from the nozzles. Replace he gDNA Filter Plate on the manifold.
6. Apply vacuum for 10 minutes to allow membrane drying. Release the vacuum.
7. Proceed to **Eluting DNA**, next page.

Continued on next page

Purification Using the EveryPrep™ Universal Vacuum Manifold, Continued

Eluting DNA

1. Prepare the EveryPrep™ Universal Vacuum Manifold for elution: Place the Elution Block and 96 Deep Well Block in the Elution Chamber, cover the top with the 96 Well Top Plate, and place the PureLink™ gDNA Filter Plate over the Top Plate.



2. Add 100–200 µl of PureLink™ gDNA Elution Buffer to the center of the membrane in each well of the gDNA Filter Plate.
3. Incubate the plate at room temperature for 1 minute.
4. Apply vacuum for 2 minutes. Release the vacuum. Disassemble the vacuum manifold to remove the elution plate.

   The purified gDNA is eluted into the elution plate.
5. Use the purified gDNA for the desired downstream application. Otherwise, cover the wells with Foil Tape, and store at 4ºC (short-term) or –20°C (long-term).

Technical Support

World Wide Web

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog

Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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MSDS Requests

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

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Purchaser Notification, Continued

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