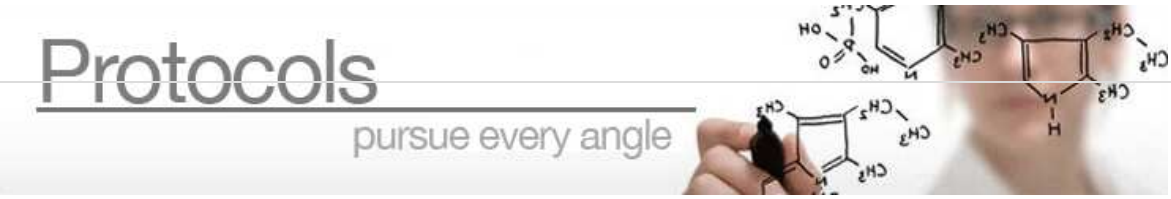
Purification of Genomic DNA Using PureLink™ Silica Columns



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Related Product Information

- PureLink™ Kits
- Quant-iT™ Technology

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Introduction

The PureLink™ Genomic DNA Purification Kit allows rapid and efficient purification of genomic DNA. The kit is designed to efficiently isolate genomic DNA from mammalian cells and tissues,mouse tail, E. coli cells, and yeast. After preparing the lysates, the DNA is purified from lysates in less than 15 minutes using a spin column based centrifugation procedure.

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

System Overview

The PureLink™ Genomic DNA Purification Kit is based on the selective binding of DNA to silica-based membrane in the presence of chaotropic salts.

The lysate is prepared from E. coli cells, yeast cells, mouse tails, and mammalian cells and tissues. The cells or tissues are digested with Proteinase K in the presence of EDTA to inhibit DNases. A detergent (SDS) is added during lysis to aid in denaturation of proteins and in solubilizing membrane proteins. The SDS also stimulates Proteinase K activity. The lysis step is performed at 55° C for E. coli, yeast, and tissues to accelerate the digestion procedure. Any residual RNA is removed by digestion with RNase prior to binding samples to the spin column.

The lysate is mixed with Binding Buffer (L3) and ethanol to adjust conditions for subsequent DNA binding to the PureLink™ Spin Column. The DNA binds to the silica-based membrane in the cartridge and impurities are removed by thorough washing with Wash Buffers. The genomic DNA is then eluted in low salt Elution Buffer (E1) or water.

Advantages

The advantages of using PureLink™ Genomic DNA Purification Kit are:

- Rapid and efficient purification of genomic DNA from a variety of samples such as mammalian cells and tissue,mouse tails, E. coli, and yeast
- Designed to purify high-quality DNA in less than 15 minutes after sample preparation
- 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

Specifications

Starting Material:	Varies
Binding Capacity:	~1 mg nucleic acid
Column Reservoir Capacity:	700 µl
Wash Tube Capacity:	2.0 ml
Centrifuge Compatibility:	Capable of centrifuging >10,000 x g
Elution Volume:	2 x 200 µl
DNA Yield:	Varies
DNA Size:	20-50 kb

Detailed protocols for preparing lysates from mammalian cells and tissues,mouse tail, E. coli, and yeast are described here. If none of the sample preparation protocols match the type or size of your sample, use the guidelines in Sample Preparation to develop your own protocol.

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Ordering Materials

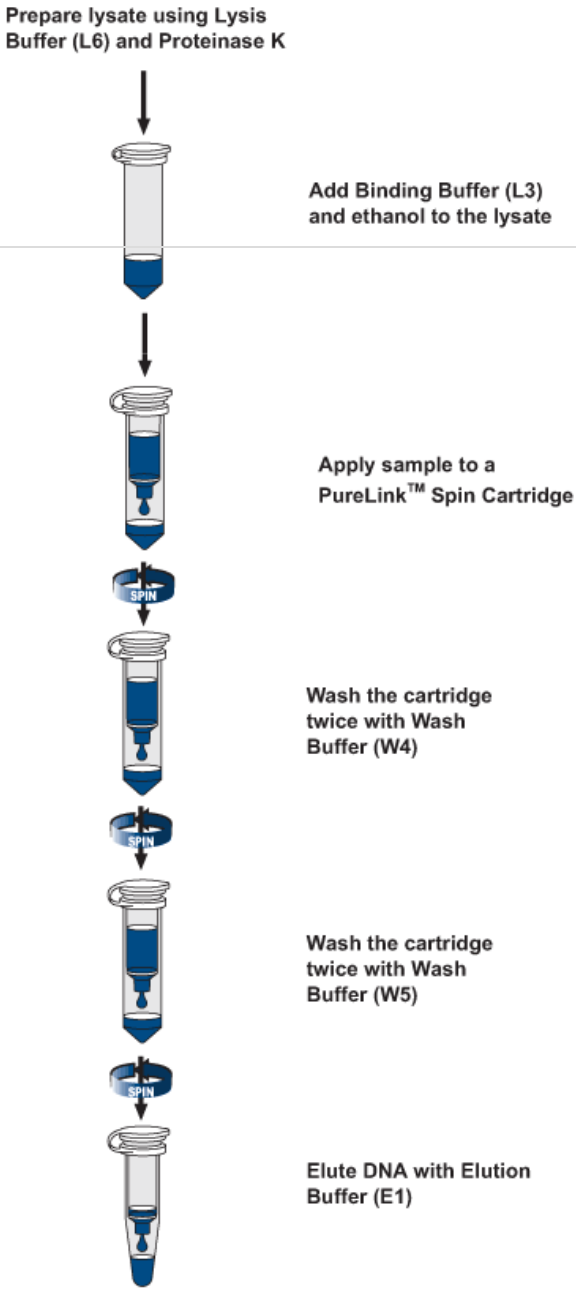
Catalog #	**Q33140**
Name	**Quant-iT™ RNA Assay Kit**
Size	1 kit
Price (USD)	**Contact Us ›**
Qty	-

Catalog #	**Q33130**
Name	**Quant-iT™ dsDNA Assay Kit, broad range**
Size	1 kit
Price (USD)	**Contact Us ›**
Qty	-

Experimental Overview

Introduction

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



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Preparing Lysates

Introduction

Instructions for preparing lysates from mammalian cells and tissues, mouse tail, bacteria, and yeast are described below.

To obtain high-quality genomic DNA, follow the guidelines recommended below.

The PureLink™ Genomic DNA Purification Kit buffers contain guanidine isothiocyanate. 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 isothiocyanate or sample preparation waste as it forms reactive compounds and toxic gases when mixed with bleach or acids.

Sample Amount

T' are different protocols for preparing lysates depending on the starting material (sample). Based on your sample, choose an appropriate lysate p .tion protocol from the table below.

The PureLink™ Genomic DNA Purification Kit is suitable for isolating DNA from a variety of samples using the recommended sample amount (see table below). If y s sample amount than the recommended amount listed in the table below, follow the appropriate protocol for the sample using the recom reagents except perform only one elution step or decrease the volume of elution buffer. **Note:** If you start with less amount of sample, the yield of DNA may also be lower.

If none of the sample preparation protocols match the type or size of your sample, then use the guidelines described below to develop your own protocol.

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.

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Sample	Amount
Mammalian cells	1-5 x 10^6 cells (suspension or adherent cells)
Mammalian tissues	Up to 25 mg
Mouse tail	0.5 cm sections
E. coli cells	Up to 2 x 10^9 cells
Yeast cells	Up to 5 x 10^7 cells

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 as an optional step during sample preparation in the protocols described in this section. The option to perform RNase digestion step will depend on the sample type and RNA content of the sample.

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, there is no need to perform the optional RNase digestion step during sample preparation. If RNA content of the sample is high (e.g., liver, kidney) and RNA contamination does interfere with any downstream applications of the purified DNA, perform the optional RNase digestion step during sample preparation.

Follow the recommendations below 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
- Be sure to mix well after addition of SDS and Binding Buffer (L3)
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples

Materials Needed

- 96-100% ethanol
- Sample for DNA isolation
- 10% SDS (To prepare 1 ml 10% SDS, mix 0.5 ml 20% SDS supplied in the kit with 0.5 ml deionized water, mix well and use)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate
- molase buffer
  - lase (lyticase) enzyme for yeast lysate
- Sterile, DNase-free microcentrifuge tubes

- Water baths or heat blocks

Compone **the kit**

- Lysis Buffer (L6)
- Binding Buffer (L3)
- Proteinase K (>400 units/ml)
- RNase (20 mg/ml)

Preparing Lysate from Mammalian Cells

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Procedure to prepare lysate from mammalian cells is described below.

1. Set a water bath or heat block at 70°C.
2. For adherent cells (up to 5 x 10^6 cells), remove the growth medium from the culture plate and harvest cells by trypsinization or a method of choice. For suspension cells (up to 5 x 10^6 cells), harvest cells and centrifuge the cells at 250 x g for 5 minutes to pellet cells. Remove the growth medium.
3. Resuspend the cells from Step 2 in 180 µl PBS.
4. Optional: Add 20 µl RNase A (supplied with the kit) to the sample and incubate at room temperature for 2 minutes.
5. Add 20 µl Proteinase K (supplied with the kit) to the sample and mix well by vortexing. Incubate at room temperature for 2 minutes.
6. Add 10 µl 10% SDS (prepared from 20% SDS supplied with the kit) to the lysate and mix immediately by vortexing for 5 seconds to denature proteins. Add 200 µl Binding Buffer (L3) supplied with the kit to the lysate. Mix well by vortexing for 5 seconds. The SDS precipitates in the presence of guanidine isothiocyanate.
7. Incubate at 70°C for 10 minutes to solubilize SDS and promote protein denaturation.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.
9. Proceed to **Binding DNA**

Preparing Mammalian Tissue Lysate

Procedure to prepare lysate from mammalian tissues is described below.

1. Set 2 water baths or heat blocks at 55°C and 70°C, respectively.
2. Place ~25 mg of minced mammalian tissue into a sterile microcentrifuge tube.
3. Add 180 µl Lysis Buffer (L6) and 20 µl Proteinase K (supplied with the kit) to the tube. Ensure the tissue is completely immersed in Lysis Buffer (L6).
   If you are using a hard mammalian tissue such as heart, you may also need to homogenize the tissue using a tissue homogenizer prior to the addition of Proteinase K.
4. Incubate at 55°C with occasional vortexing until lysis is complete (~3 hours).
5. **Optional:** Add 20 µl RNase A (supplied in the kit) to lysate and incubate at room temperature for 2 minutes.
6. Centrifuge the lysate at maximum speed for 5 minutes at room temperature to remove any particulate materials.
7. Transfer the supernatant to a fresh microcentrifuge tube, add 10 µl 10% SDS (prepared from 20% SDS supplied with the kit) to the lysate and mix immediately by vortexing for 5 seconds to denature proteins. Add 200 µl Binding Buffer (L3) supplied with the kit to the lysate. Mix well by vortexing for 5 seconds. The SDS precipitates in the presence of guanidine isothiocyanate.
8. Incubate at 70°C for 10 minutes to solubilize SDS and promote protein denaturation.
9. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.
10. Proceed to **Binding DNA**

Preparing Lysate from Mouse Tail

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Procedure to prepare lysate from mouse tail is described below. **Note:** The sample preparation protocol may not require any RNase A treatment step as mouse tails contain low levels of RNA.

1. Set 2 water baths or heat blocks at 55°C and 70°C, respectively.

2. Cut the mouse tail in 0.5 cm pieces. Place one 0.5 cm piece into a sterile microcentrifuge tube and cut the tail piece into small pieces.
3. Add 180 µl Lysis Buffer (L6) and 20 µl Proteinase K (supplied with the kit) to the tube and mix well. Ensure the pieces are completely immersed in buffer.
4. Incubate at 55°C with occasional vortexing until lysis is complete (~6-8 hours). You can also perform the lysis overnight.
5. Add 20 µl RNase A (supplied in the kit) to lysate and incubate at room temperature for 2-5 minutes.
6. Centrifuge the lysate at maximum speed for 5 minutes at room temperature to remove any hair from the lysate.
7. Transfer the supernatant to a fresh microcentrifuge tube, add 10 µl 10% SDS (prepared from 20% SDS supplied with the kit) to the lysate and mix immediately by vortexing for 5 seconds to denature proteins. Add 200 µl Binding Buffer (L3) supplied with the kit to the lysate. Mix well by vortexing for 5 seconds. The SDS precipitates in the presence of guanidine isothiocyanate
8. Incubate at 70°C for 10 minutes to solubilize SDS and promote protein denaturation.
9. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.
10. Proceed to **Binding DNA**.

Preparing E. coli Lysate

Procedure to prepare E. coli cell lysate is described below.

1. Set 2 water baths or heat blocks at 55°C and 70°C, respectively.
2. Harvest up to 2 x 10^9 E. coli cells by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
3. Resuspend the cell pellet in 180 µl Lysis Buffer (L6) supplied with the kit. Add 20 µl Proteinase K solution (supplied with the kit) to lyse the cells. Mix well by vortexing for 5 seconds.
4. Incubate the tube at 55°C for 15 minutes.
5. **Optional:** Add 20 µl RNase A (supplied with the kit) to lysate and incubate at room temperature for 2 minutes.
6. Add 10 µl 10% SDS (prepared from 20% SDS supplied with the kit) to the lysate and mix immediately by vortexing for 5 seconds to denature proteins. Add 200 µl Binding Buffer (L3) supplied with the kit to the lysate. Mix well by vortexing for 5 seconds. The SDS precipitates in the presence of guanidine isothiocyanate.
7. Incubate at 70°C for 10 minutes to solubilize SDS and promote protein denaturation.
8. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.
9. Proceed to **Binding DNA.**

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Preparing Lysate from Yeast Cells

Procedure to prepare lysate from yeast cells is described below.

1. Set 3 water baths or heat blocks at 37°C, 55°C, and 70°C, respectively.
2. Prepare fresh 10 ml Zymolase Buffer (1 M sorbitol, 10 mM sodium EDTA, 14 mM ß-mercaptoethanol).
3. Harvest up to 5 x 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 prepared as described above. Add 15 units zymolase (lyticase) enzyme and incubate at 37° C for 1 hour to generate spheroplasts.
5. Centrifuge at 3000 x g for 10 minutes at room temperature to pellet the spheroplasts.
6. Resuspend the spheroplasts in 180 µl Lysis Buffer (L6) supplied with the kit. Add 20 µl Proteinase K (supplied with the kit). Mix well by vortexing for 5 seconds.
7. Incubate the tube at 55°C for 45 minutes.
8. Optional: Add 20 µl RNase A (supplied in the kit) to the lysate and incubate at room temperature for 2 minutes.
9. Add 10 µl 10% SDS (prepared from 20% SDS supplied with the kit) to the lysate and mix immediately by vortexing for 5 seconds to denature proteins. Add 200 µl Binding Buffer (L3) supplied with the kit to the lysate. Mix well by vortexing for 5 seconds. The SDS precipitates in the presence of guanidine isothiocyanate.
10. Incubate at 70°C for 10 minutes to solubilize SDS and promote protein denaturation.
11. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.
12. Proceed to **Binding DNA**

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 Lysis Buffer (L6) 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 Binding Buffer (L3) and 96-100% ethanol prior to loading the sample onto the column. Always maintain a ratio of 1:1:1 for Lysis Buffer:Binding Buffer:Ethanol to obtain optimal binding.

neral protocol for lysate preparation can be as follows:

For cells, resuspend cell pellet in 180 µl Lysis Buffer (L6) 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 Lysis Buffer (L6). 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. **Optional:** 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 and add 10 µl 10% SDS (prepared from 20% SDS supplied with the kit) to the lysate. Add 200 µl Binding Buffer (L3) supplied with the kit to the lysate. Mix well by vortexing for 5 seconds. The SDS precipitates in the presence of guanidine isothiocyanate.
5. Incubate at 70°C for 10 minutes to solubilize SDS and promote protein denaturation.
6. Add 200 µl 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.
7. Proceed to **Binding DNA.**

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Purification Procedure

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

- Lysates prepared as described above
- Sterile, DNase-free 1.5 ml microcentrifuge tubes for elution
- Microcentrifuge capable of centrifuging >10,000 x g
- **Optional:** sterile water, pH 7-8.5, if you are using water for elution

Components supplied with the kit

- Wash Buffers (W4) and (W5)
- Elution Buffer (E1)
- PureLink™ Spin Cartridge in Collection Tubes
- Wash Tubes

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Perform a 1 minute incubation step with Elution Buffer (E1) or water
- Be sure to perform the recommended wash steps to obtain the best results
- Always use sterile water, pH 7-8.5, if you are using water for elution

Elution Volume

The DNA is eluted in 2 aliquots of 200 µl each to obtain higher DNA yield. The DNA recovery in the first elution is 65-80% and after second elution is >95%.

To prevent dilution of the DNA sample and also avoid contact of the spin column with the eluate, perform the two-elution steps using different tubes.

Before Starting

Add 40 ml 96-100% ethanol to 10 ml Wash Buffer (W5) included with the kit. Store the Wash Buffer (W5) with ethanol at room temperature.

Binding DNA

1. Remove a PureLink™ Spin Cartridge in a Collection Tube from the package.
2. Add the [redacted] buffer (L3) and ethanol prepared to the PureLink™ Spin Cartridge.
3. Centrifuge the cartridge at 12,000 x g for 30 seconds at room temperature.
4. Discard the collection tube and place the spin cartridge into a clean Wash Tube supplied with the kit.
5. Proceed to Washing DNA

Washing DNA

1. Add 500 µl Wash Buffer (W4) supplied in the kit to the cartridge.
2. Centrifuge cartridge at room temperature at 12,000 x g for 30 seconds. Discard flow through from the Wash Tube and place cartridge into the Wash Tube.
3. Repeat Steps 1-2, once. Discard the flow through.
4. Add 500 µl Wash Buffer (W5) with ethanol (page 13) to the cartridge.
5. Centrifuge the cartridge at 12,000 x g for 30 seconds at room temperature. Discard the flow through from the Wash Tube and place the cartridge into the Wash Tube.
6. Repeat Steps 4-5, once. Discard the flow through.
7. Centrifuge the cartridge at maximum speed for 2 minutes at room temperature to remove any residual Wash Buffer (W5). Discard Wash Tube.
8. Proceed to Eluting DNA.

Eluting DNA

1. Place the spin cartridge in a sterile 1.5-ml microcentrifuge tube.
2. Add 200 µl of Elution Buffer (E1) or sterile, distilled water (pH >7.0) to the cartridge.
3. Incubate at room temperature for 1 minute. Centrifuge the cartridge at maximum speed for 1.5 minute at room temperature. The tube contains purified DNA.
4. To recover more DNA, perform a second elution step with 200 µl Elution Buffer (E1) or sterile, distilled water (pH >7.0) using another sterile 1.5 ml microcentrifuge tube.
5. Centrifuge the column at maximum speed for 1.5 minute at room temperature. The tube contains purified DNA. Remove and discard the cartridge.

Based on the volume of elution buffer used for elution, the recovery of the elution volume varies and is usually >95% of the elution buffer volume used.

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 Elution Buffer (E1) 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.

The Next Step

You may determine the quality, quantity, and length of the purified DNA as described in Analyzing DNA Yield and Quality.

Genomic DNA isolated using the PureLink™ Genomic DNA Purification Kit is suitable for use in any downstream application of choice.

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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.
2. Calculate the amount of DNA using the formula:

DNA (µg) = A_{260} x 50 µg/(1 A_{260} x 1 ml) x dilution factor x 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.

Note: Any contamination from RNA will inflate the DNA content measured at 260 nm. To avoid any interference from RNA, use the Quant-iT™ Kits.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits 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.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

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 PFGE (Pulse-Field Gel Electrophoresis) on an agarose gel.

The DNA isolated using the PureLink™ Genomic DNA Purification 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.

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Expected Results

Introduction

The DNA yield obtained from various samples is described below.

DNA Yield

The yield of genomic DNA obtained from various samples using the PureLink™ Genomic DNA Purification Kit is listed below: The DNA quantitation was performed with the Quant-iT™ DNA Assay Kits (see above).. The yield is the total yield from 2 x 200 µl elution.

Material	Amount	DNA Yield
E. coli cells	1 x 10^9	5-15 µg
HeLa cells	1 X 10^6	5-10 µg
lls	1 x 10^6	2-6 µg

Huh-7 cells	2 x 10^6	3-10 µg
Mouse tail	0.5 cm	4-9 µg
Mouse liver	25 mg	5-8 µg

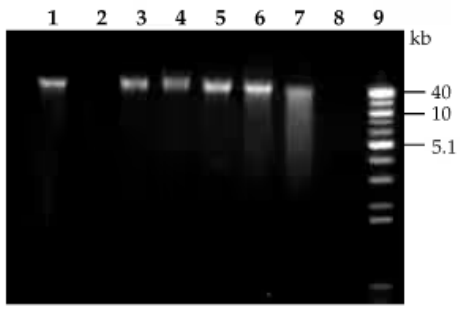
Note: The DNA yield varies with the sample and DNA content of the sample

DNA Quality

Genomic DNA isolated from various samples was analyzed by agarose gel electrophoresis on a 0.8% E-Gel® agarose gel.

Samples on the gel are:

Lane 1	36.7 ng of DNA isolated from E. coli (5 x 10^8 cells)
Lane 2	Blank
Lane 3	21.8 ng DNA isolated from human HeLa (1x10^6 cells)
Lane 4	19.3 ng DNA isolated from human Huh-7 (2x10^6 cells)
Lane 5	37.5 ng DNA isolated from human 293F (1x10^6 cells)
Lane 6	60.3 ng DNA isolated from mouse liver (25 mg)
Lane 7	69.1 ng DNA isolated from mouse tail (0.5-cm section)
Lane 8	Blank
Lane 9	1 Kb DNA Extension Ladder (0.5 µg/lane)



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Troubleshooting

Introduction

Refer to the table below to troubleshoot any problems you may encounter with the PureLink™ Genomic DNA Purification Kit.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	Decrease the amount of starting material used. Be sure to add Proteinase K and SDS solution during lysis. For tissues, cut the tissue into smaller pieces and ensure the tissue is completely immersed in the Lysis Buffer to obtain optimal lysis. If incomplete lysis is observed, increase the incubation 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 is clogged	Make sure that the lysate is clear when the lysate is loaded on to the spin cartridge. Remove any particulate or viscous material by centrifugation prior to loading the lysate on to the spin cartridge.
	Incorrect binding conditions	Be sure to add Binding Buffer (L3) and 96-100% ethanol to the lysate prior to loading the samples on the spin cartridge. Mix the sample properly with Binding Buffer and ethanol by vortexing. Avoid overloading the cartridge.
	Ethanol not added to Wash Buffer (W5)	Be sure to add 96–100% ethanol to Wash Buffer (W5).
	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 extensive pipetting to facilitate lysis/homogenization and repeated freezing and thawing of samples to prevent any DNA damage. Maintain a sterile environment while working to avoid any contamination from DNases.
Dark colored eluate or discolored membrane (mammalian tissue or mouse tails only)	Pigments from tissues bind to the cartridge matrix and co-elute with DNA	Be sure to add ethanol to the lysate prior to loading the lysate on to the spin cartridge. The ethanol prevents the pigments from sticking on the cartridge matrix. Perform centrifugation of the lysate at a higher speed and longer time prior to loading the lysate on to the cartridge.
RNA contamination	--	Perform optional RNase digestion step during the sample preparation.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Traces of ethanol from the Wash Buffer (W5) can inhibit downstream enzymatic reactions. To remove Wash Buffer (W5), discard Wash Buffer (W5) flow through. Place the spin cartridge into the Wash Tube and centrifuge the spin cartridge at maximum speed for 2-3 minutes to completely dry the cartridge.
	Presence of salt in purified DNA	Use the correct order of Wash Buffers for washing. Always wash the cartridge with Wash Buffer (W4) followed by washing with Wash Buffer (W5).

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