

iPrep[™] ChargeSwitch[®] gDNA Tissue Kit

**For purification of genomic DNA (gDNA) from
tissue using the iPrep[™] instrument**

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Experienced Users Procedure

Introduction

This quick reference sheet is included for experienced users of the iPrep™ ChargeSwitch® gDNA Tissue Kit. For more details, refer to this manual.

Step	Procedure
Prepare tissue lysate	<ol style="list-style-type: none">1. Grind a small piece of tissue with liquid nitrogen into a fine, homogenous powder using a mortar and pestle.2. Place up to 10 mg of powdered tissue (above) or up to 10 mg frozen tissue into a sterile glass homogenizer tube. Add 1 mL Lysis Buffer (L13) and homogenize using 8–10 strokes or until tissue is completely homogenized.3. Transfer the homogenized tissue to a sterile microcentrifuge tube. Add 10 µL RNase A to the lysate and vortex briefly.4. Incubate for 10 minutes at room temperature.5. Add 20 µL Proteinase K. Mix by vortexing and incubate the sample at 55°C until lysis is complete (1–3 hours).6. Allow the sample to cool and vortex the sample. The lysate should be almost clear and homogenous without any particulate material.
Purification Protocol	<ol style="list-style-type: none">1. Open the iPrep™ Card Slot and insert the iPrep™ Card: gDNA Tissue in the slot (arrow on the card is at the top and card label is facing your left side).2. Turn ON the iPrep™ Instrument using the power switch on the left side of the instrument. The digital display shows the version for the iPrep™ instrument which changes in few seconds to display the Main menu.3. Press Start to run a protocol.4. Press 1 to select the Tissue protocol.5. Open the iPrep™ instrument door and remove iPrep™ Racks to set up the platform.6. Remove the iPrep™ Tissue Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.

Continued on next page

Experienced Users Procedure, Continued

Step	Procedure
Purification Protocol, Continued	<ol style="list-style-type: none"> 7. Load the desired number of cartridges on the iPrep™ Cartridge Rack and insert the rack on the iPrep™ platform. 8. Load the iPrep™ Tip and Tube Rack as follows: <ul style="list-style-type: none"> • Load the first row (labeled as E) with 1–13 elution tubes without caps • Keep second row (labeled as T1) empty • Load the third row (labeled as T2) with iPrep™ Tips in the iPrep™ Tip Holders • Load the fourth row (labeled as S) with sample tubes without caps and add the lysate (~1 mL from Step 6, previous page) to the sample tubes 10. Read the sample and elution tube barcodes, if needed. 11. Insert the iPrep™ Tip and Tube Rack on to the iPrep™ Platform and close the iPrep™ instrument door. 12. Press 1 to continue. 13. Press Start. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display. 14. At the end of the run, the instrument beeps briefly and the digital display shows Protocol Finished for 10 seconds. The Main menu appears after 10 seconds. 15. Open the instrument door. 16. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified gDNA at 4°C (short-term) or aliquot and store at –20°C (long-term). 17. Discard the used cartridges, tips, and sample tubes into biohazard waste. Do not reuse the cartridges. 18. To purify more samples using the same iPrep™ Protocol Card, load the racks with new cartridges, tips, and samples, and start the protocol as described above. 19. If you are not using the instrument, close the instrument door and turn the power switch to OFF. 20. Remove the iPrep™ Card and store the card in the box.

Kit Contents and Storage

Shipping and Storage

Each iPrep™ ChargeSwitch® gDNA Tissue Kit contains Reaction Cartridges and disposable plasticware (Box 1) and sample preparation reagents (Box 2).

Upon receipt, store Box 1 at room temperature and Box 2 at 4°C. See below for kit contents.

All components are guaranteed stable for 6 months when stored properly.

Box 1 Contents

The components supplied in the iPrep™ ChargeSwitch® gDNA Tissue Kit (Box 1) are listed below.

Store Box 1 at room temperature. Do not freeze the iPrep™ Tissue Cartridge Kit.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
iPrep™ Tissue Cartridge Kit	1 kit
iPrep™ Sample and Elution Tubes	2 × 52 tubes
iPrep™ Tips and Tip Holders	1 bag with 52 tips and holders

Continued on next page

Kit Contents and Storage, Continued

iPrep™ Tissue Cartridge Kit Contents

Each iPrep™ Tissue Cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube).

Positions 1–6 contain wells filled with reagents while positions 7–10 contain empty wells for this protocol.

The components supplied in each well of the iPrep™ Tissue Cartridge Kit (Box 1) are listed below.

Do not freeze the iPrep™ Tissue Cartridge Kit.

Reagent	Well no.
ChargeSwitch® Magnetic Beads (27 mg/mL in 10 mM potassium acetate, pH 4.0, 6.6 mM KCl)	1
ChargeSwitch® Purification Buffer (N5)	2
ChargeSwitch® Wash Buffer (W12)	3
ChargeSwitch® Wash Buffer (W16)	4
ChargeSwitch® Wash Buffer (W12)	5
ChargeSwitch® Elution Buffer (E5, 10 mM Tris-HCl, pH 8.5)	6
Empty	7–10

Box 2 Contents

The components supplied in the iPrep™ ChargeSwitch® gDNA Tissue Kit (Box 2) are listed below.

Store Box 2 at 4°C.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
Proteinase K (20 mg/mL in 50 mM Tris-HCl, pH 8.0, 5 mM CaCl ₂ , 50% glycerol)	2 × 550 µL
ChargeSwitch® Lysis Buffer (L13)	52 mL
RNase A (5 mg/mL in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	520 µL

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Introduction

Product Overview

Introduction

The iPrep™ ChargeSwitch® gDNA Tissue Kit allows rapid, automated purification of genomic DNA from a variety of tissue samples using the ChargeSwitch® Technology with the iPrep™ Purification Instrument.

After preparing the lysates, DNA is purified within 30 minutes. The purified genomic DNA is suitable for use in downstream applications including PCR, restriction enzyme digestion, and Southern blotting.

For more information about the ChargeSwitch® Technology, see page 3.

iPrep™ Purification Instrument

The iPrep™ ChargeSwitch® gDNA Tissue Kit is designed for use with the iPrep™ Purification Instrument.

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe unit capable of purifying nucleic acids from up to 13 samples (12 samples + 1 positive control) using a magnetic bead-based technology. See page 4 for details on the iPrep™ Purification Instrument.

System Overview

The iPrep™ ChargeSwitch® gDNA Tissue Kit combines the powerful ChargeSwitch® technology (page 3) with the speed and convenience of the iPrep™ Instrument to allow automated purification of high-quality DNA from up to 13 samples within 30 minutes. Purification is achieved using a simple bind–wash–elute procedure, and avoids the use of guanidine, ethanol, and other chaotropic reagents.

The lysate is prepared from tissue samples using Proteinase K at 55°C to accelerate the digestion procedure. Any residual RNA is removed by digestion with RNase A.

The lysate is mixed with ChargeSwitch® Magnetic Beads for subsequent DNA binding to the beads. The DNA-bound magnetic beads are separated from the lysate using magnetic separation. The beads are thoroughly washed with Wash Buffers to remove contaminants. The genomic DNA is then eluted in Elution Buffer (E5).

Continued on next page

Product Overview, Continued

Advantages	<p>Use of the iPrep™ ChargeSwitch® gDNA Tissue Kit to isolate genomic DNA provides the following advantages:</p> <ul style="list-style-type: none">• Uses a magnetic bead-based technology to isolate genomic DNA without the need for hazardous chemicals, centrifugation, or vacuum manifolds• Rapid and automated purification of genomic DNA from up to 10 mg tissue samples in less than 30 minutes following sample lysis using the iPrep™ Instrument• Pre-filled reagent cartridges provide easy set up and consistent results• Minimal contamination with RNA• The purified genomic DNA demonstrates improved downstream performance in applications including PCR, restriction enzyme digestion, and Southern blotting.
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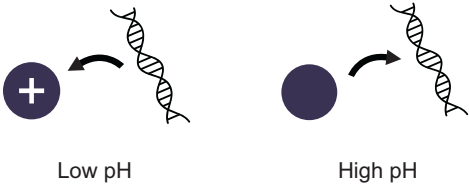
System Specifications	<table><tr><td>Starting Material:</td><td>Up to 10 mg</td></tr><tr><td>Sample Type:</td><td>Various animal tissue samples</td></tr><tr><td>Number of Samples:</td><td>Up to 13</td></tr><tr><td>Elution Volume:</td><td>200 µL</td></tr><tr><td>DNA Yield:</td><td>Up to 12 µg</td></tr><tr><td>DNA Size:</td><td>At least 40 kb</td></tr></table>	Starting Material:	Up to 10 mg	Sample Type:	Various animal tissue samples	Number of Samples:	Up to 13	Elution Volume:	200 µL	DNA Yield:	Up to 12 µg	DNA Size:	At least 40 kb
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Sample Type:	Various animal tissue samples												
Number of Samples:	Up to 13												
Elution Volume:	200 µL												
DNA Yield:	Up to 12 µg												
DNA Size:	At least 40 kb												

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Product Overview, Continued

The ChargeSwitch[®] Technology

The ChargeSwitch[®] Technology (CST[®]) is a novel magnetic bead-based technology that provides a switchable surface charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification. In low pH conditions, the CST[®] beads have a positive charge that binds the negatively charged nucleic acid backbone (see figure below). Proteins and other contaminants are not bound and are simply washed away in an aqueous wash buffer. To elute nucleic acids, the charge on the surface of the bead is neutralized by raising the pH to 8.5 using a low salt elution buffer (see figure below). Purified nucleic acid elutes instantly into this elution buffer, and is ready for use in downstream applications.



ChargeSwitch[®] Magnetic Bead Specifications

Bead Binding Capacity:	5–10 µg genomic DNA per mg
Bead Size:	<1 µm
Bead Concentration:	27 mg/mL
Storage Buffer:	10 mM potassium acetate, pH 4.0, 6.6 mM KCl

iPrep™ Purification Instrument

Introduction

The iPrep™ Purification Instrument is a benchtop, automated nucleic acid purification instrument with integrated Magnetic and Syringe unit capable of purifying nucleic acids from up to 12 samples and one positive control. Each iPrep™ Instrument consists of the Magnetic and Syringe Unit, and a platform. A pre-programmed iPrep™ Card controls the purification parameters such as buffer volumes, mixing steps, and incubation time.

For more details on the iPrep™ Purification Instrument, see the manual supplied with the instrument.

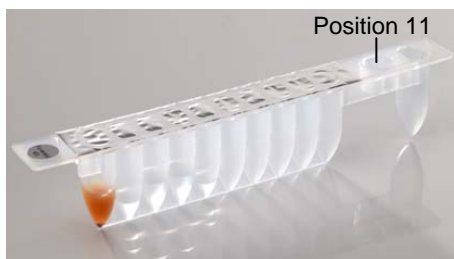
iPrep™ Reaction Cartridge

The iPrep™ Reaction Cartridges are supplied with the iPrep™ Kits and are designed to fit onto the iPrep™ Cartridge Rack in only one orientation. Each cartridge is pre-filled with reagents required for the iPrep™ ChargeSwitch® gDNA Tissue protocol. Each cartridge has 12 positions with 10 sealed wells and two heating positions (position 12 with an empty well and position 11 to add an empty or reagent filled tube). For the iPrep™ ChargeSwitch® gDNA Tissue Kit, positions 1–6 contain wells filled with reagents and wells 7–10 are empty.

Cartridge Specifications:

Material:	Polypropylene cartridge sealed with laminated aluminum foil
Max Volume:	1,000 µL/well
Dimension:	5.9 inches (l) × 1.2 inches (w) × 0.7 inches (d)

Note: The image below shows an example of an iPrep™ Reagent Cartridge and is not an image of an iPrep™ Tissue Cartridge.



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iPrep™ Purification Instrument, Continued

iPrep™ Tips and Tip Holders

The iPrep™ Tips and Tip Holders are included with the iPrep™ Kits and are placed on the iPrep™ Tip and Tube Rack as described on page 13. While assembling the tips on the rack, always insert the iPrep™ Tips into the iPrep™ Tip Holders using gloved hands. Always use the tips with the holders to prevent any contamination.

Tip Specifications:

Tip Material:	Polypropylene with filter barriers
Tip Holder Material:	Polypropylene
Volume:	5–1,000 µL
Tip Dimension:	3.9 inches (l) × 0.43 inches (d)

iPrep™ Tip Holder



iPrep™ Tip



iPrep™ Tubes

Two sets of iPrep™ Tubes are required for the purification protocol. The iPrep™ Sample and Elution Tubes are included with each iPrep™ Kit and placed on the iPrep™ Tip and Tube Rack as described on page 13.

Tube Specifications:

Material:	Polypropylene
Capacity:	1.5 mL
Style:	Tubes with caps
Dimensions:	1.7 inches (l) × 0.4 inches (d)



Continued on next page

iPrep™ Purification Instrument, Continued

iPrep™ Card: gDNA Tissue

To use the iPrep™ ChargeSwitch® gDNA Tissue Kit with the iPrep™ Purification Instrument, you need to purchase the iPrep™ Card: gDNA Tissue (page 19).

The iPrep™ Card: gDNA Tissue is pre-programmed with the tissue purification protocol that directs the volume of reagents used and incubation time.

Always store the card in the box, protected from light.

To avoid damaging the card:

- Do not drop or bend the card
- Do not wipe or clean the card using volatile chemicals such as alcohol or equivalent
- Do not expose the card to water

iPrep™ Platform

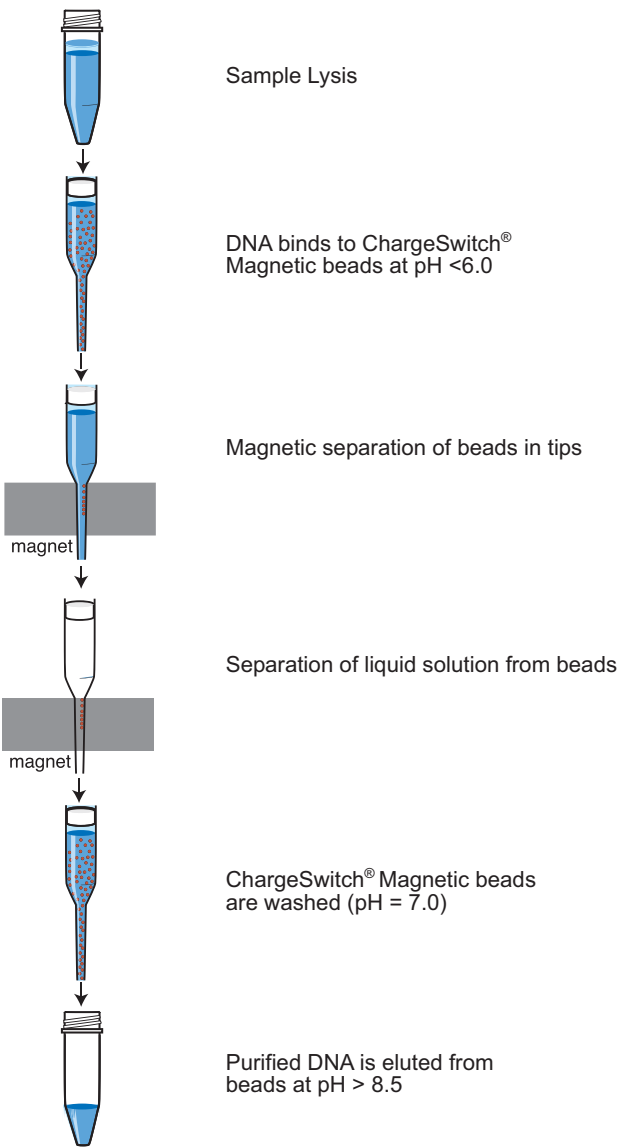
The platform on the iPrep™ Instrument allows the placement of iPrep™ Tip and Tube Rack, and iPrep™ Cartridge Rack that are filled with plastic disposables and reagent cartridges required for the purification protocol.

Set up the platform as shown in the figure on page 13 for the iPrep™ ChargeSwitch® gDNA Tissue Kit.

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to purify genomic DNA from tissues using the iPrep™ ChargeSwitch® gDNA Tissue Kit.



Methods

General Information

User Supplied Materials

In addition to the reagents supplied with the kit, you also need the following materials and instrumentation:

- iPrep™ Purification Instrument (page 19)
- iPrep™ Card: gDNA Tissue (page 19)
- Sterile, 1.5 mL tubes or you may use iPrep™ Tubes
- Mortar and pestle
- Glass homogenizer or equivalent
- Tissue sample (page 10)



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
- Do not vortex the samples for more than 5–10 seconds during sample lysis to avoid extensive shearing of DNA
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples
- Do not freeze the beads as this irreparably damages them. Store the beads at room temperature.
- When using the beads from the Reaction Cartridges, collect any solution from the foil by tapping the cartridge to deposit the solution at the bottom of the tube. Do not allow the beads to dry out as this renders them non-functional.
- Discard Reaction Cartridges, iPrep™ Tips, and iPrep™ Tip Holders after use. Do not reuse.

Continued on next page

General Information, Continued

Safety Information

Follow the safety guidelines below when using the iPrep™ ChargeSwitch® gDNA Tissue Kit.

- Treat all reagents supplied in the kit as potential irritants.
 - Always wear a suitable lab coat, disposable gloves, and protective goggles.
 - If a spill of the buffers in the cartridges occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
-

Isolating Genomic DNA

Introduction

Instructions to isolate genomic DNA from tissue samples using the iPrep™ ChargeSwitch® gDNA Tissue Kit with the iPrep™ Purification Instrument are described below.

Starting Material

Use this procedure to isolate genomic DNA from up to 10 mg of animal tissue samples.

Materials Needed

You will need the following materials:

- Tissue samples (see above)
- iPrep™ Purification Instrument (page 19)
- iPrep™ Card: gDNA Tissue (page 19)
- Mortar and pestle
- 1 mL Glass homogenizer or equivalent
- Water bath or heat block set to 55°C

Components Supplied with the Kit

- iPrep™ Tissue Cartridge Kit
 - iPrep™ Sample and Elution Tubes
 - iPrep™ Tips
 - iPrep™ Tip Holders
 - Proteinase K
 - RNase A
 - ChargeSwitch® Lysis Buffer (L13)
-

Before Starting

Perform the following before starting:

- Set a water bath or heat block at 55°C
 - Ensure you have the iPrep™ Card: gDNA Tissue (page 19) to run the tissue protocol
 - Make sure the iPrep™ Purification Instrument is unpacked and installed
-

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Isolating Genomic DNA, Continued

Preparing the Tissue Lysate

Instructions are included to prepare the lysate using powdered tissue or frozen tissue.

To standardize and normalize the amount of tissue used for each sample, and obtain consistent results, we recommend using powdered tissue to prepare the lysate. Choose an appropriate protocol based on your needs.

Powdered Tissue

1. Place a piece of tissue in a mortar and pestle and add a small volume of liquid nitrogen. Grind the tissue into a fine, homogenous powder using the mortar and pestle.
2. Transfer the powdered tissue into a 50 mL conical tube. Keep the tube open to allow the liquid nitrogen to evaporate. Cap the tube and store at -80°C .
3. Place up to 10 mg of ground tissue (Step 2) into a sterile glass homogenizer tube. Proceed to **Preparing Lysate**, below.

Frozen Tissue

Place up to 10 mg frozen tissue sample into a sterile glass homogenizer tube. Proceed to **Preparing Lysate**, below.

Preparing Lysate

1. Add 1 mL of Lysis Buffer (L13) to the powdered or frozen tissue sample in the tube. Homogenize until the tissue is completely homogenized.
2. Transfer the homogenized tissue to a sterile microcentrifuge tube. Add 10 μL RNase A to the lysate and vortex briefly.
3. Incubate for 10 minutes at room temperature.
4. Add 20 μL Proteinase K. Mix by vortexing and incubate the sample at 55°C until lysis is complete (1–3 hours).

Note: Vortex the sample at least 4–5 times during this period.

5. Allow the sample to cool and vortex the sample. The lysate should be almost clear and homogenous without any particulate material.

Note: If you notice any particulate material, centrifuge the lysate at maximum speed for 5 minutes at room temperature to remove any particulate materials.

6. Proceed to **Purification Protocol**, next page.

Continued on next page

Isolating Genomic DNA, Continued

Purification Protocol

Purify genomic DNA from the lysate using the iPrep™ Purification Instrument as described below.

For details on using the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

Insert the iPrep™ Card: gDNA Tissue (available separately from Invitrogen, page 19) prior to turning on the instrument.

1. Ensure the power switch on the iPrep™ Instrument is on the **OFF** position.
2. Open the iPrep™ Card Slot and insert the iPrep™ Card: gDNA Tissue into the slot in the correct orientation (arrow on the card is at the top and card label is facing your left side).
3. Using the power switch located on the left side of the instrument, turn **ON** the instrument.

If the card is fully inserted in the correct orientation, all axes return to their original positions automatically. The digital display shows the version for the iPrep™ instrument which changes in a few seconds to display the Main menu.

4. Press **Start** to run a protocol.
5. Press **1** to select the Tissue protocol.
6. Open the iPrep™ door and remove the iPrep™ Cartridge Rack and iPrep™ Tip and Tube Rack to set up the platform.
7. Remove the iPrep™ Tissue Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.
8. Load the desired number of cartridges on the iPrep™ Cartridge Rack.

Note: You can load 1–13 cartridges on the rack depending on the number of samples that you wish to process. If you are loading less than 13 cartridges, ensure that the remaining plastic ware (tips and tubes) are also loaded in the same order as the cartridges.

Continued on next page

Isolating Genomic DNA, Continued

Purification Protocol, Continued

Procedure continued from previous page

9. Load the iPrep™ Tip and Tube Rack as follows (see figure below):
 - Load the first row (labeled as **E**) with 1–13 elution tubes **without caps** (you may place the caps on the rack as shown in the figure below)
 - Keep the second (labeled as **T1**) row empty
 - Load the third row (labeled as **T2**) with iPrep™ Tips in the iPrep™ Tip Holders.
 - Load the fourth row (labeled as **S**) with sample tubes **without caps** and add the lysate (~1 mL from Step 5, page 11) to the sample tubes



10. Read the sample and elution tube barcodes, if needed.
11. Insert the iPrep Tip and Tube rack on the iPrep™ platform as shown above.
12. Close the iPrep™ instrument door.

Continued on next page

Isolating Genomic DNA, Continued

Purification Protocol, Continued

Procedure continued from previous page

13. Press **1** to continue.
14. Ensure that you have loaded the cartridges, tubes, and tips in the appropriate positions, and the sample and elution tubes **do not have any caps**.
15. Press **Start**. The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display.

Important: Do not open the door once the protocol has begun.

To pause the protocol, press the **Stop** key. To resume the protocol after a pause, press the **Start** key. To cancel/stop the protocol, press the **Stop** key twice. For details, see the iPrep™ Instrument manual.
16. At the end of the run, the instrument beeps briefly and the digital display shows **Protocol Finished** for 10 seconds. The Main menu appears after 10 seconds.
17. Open the instrument door. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified gDNA as described below.
18. Discard the used cartridges, tips, and sample tubes into biohazard waste. Do not reuse the cartridges.
19. To purify more samples using the same iPrep™ protocol Card, load the racks with new cartridges, tips, and samples, and start the protocol as described above.
20. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**.
21. Remove the iPrep™ Card and store the card in the box, protected from light.

Storing DNA

- Use the purified DNA immediately for the desired downstream application.
 - Store the purified DNA at 4°C for short-term use or aliquot the DNA and store at -20°C for long-term storage to avoid repeated freezing and thawing of purified DNA.
-

DNA Quantitation and Analysis

Quantitating DNA Yield

You may estimate the yield of purified genomic DNA by checking the UV absorbance at 260 nm or using one of the 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 8.5.
2. Calculate the amount of DNA using the formula:
$$\text{DNA } (\mu\text{g}) = A_{260} \times 50 \mu\text{g} / (A_{260} \times 1 \text{ mL}) \times \text{dilution factor} \times \text{total sample volume (mL)}$$

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

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (see page 19 for ordering information) provide a rapid, sensitive, and accurate 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, pre-diluted standards for a standard curve, and a pre-made buffer to allow fluorescence-based DNA quantitation. For more information, see www.invitrogen.com or call Technical Support (page 20).

Analyzing DNA Quality

Typically, DNA isolated using the iPrep™ ChargeSwitch® gDNA Tissue 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.

Purified gDNA may be analyzed by agarose gel electrophoresis to check the DNA quality (usually a single band at >40 kb with no smearing) and confirm the absence of contaminating RNA.

Genomic DNA isolated with the iPrep™ ChargeSwitch® gDNA Tissue Kit is usually >40 kb in size.

Note: The size usually varies and is dependent on the quality of the starting material.

Expected Results

Example of DNA Yield

The yield of genomic DNA obtained from 10 mg of various tissue samples using the iPrep™ ChargeSwitch® gDNA Tissue Kit is listed below.

The DNA quantitation was performed with the Quant-iT™ PicoGreen® dsDNA Assay Kit.

Material	DNA Yield	UV A _{260/280 nm} Ratio
Rat lung	11.9 µg	1.84
Rat heart	7.0 µg	1.85
Rat kidney	11.9 µg	1.82
Rat spleen	5.5 µg	1.85
Rat liver	11.4 µg	1.83

Example of DNA Quality

Genomic DNA (10 µL) isolated from various tissue samples was analyzed by agarose gel electrophoresis on a 1% E-Gel® 48 gel.

Samples on the gel are:

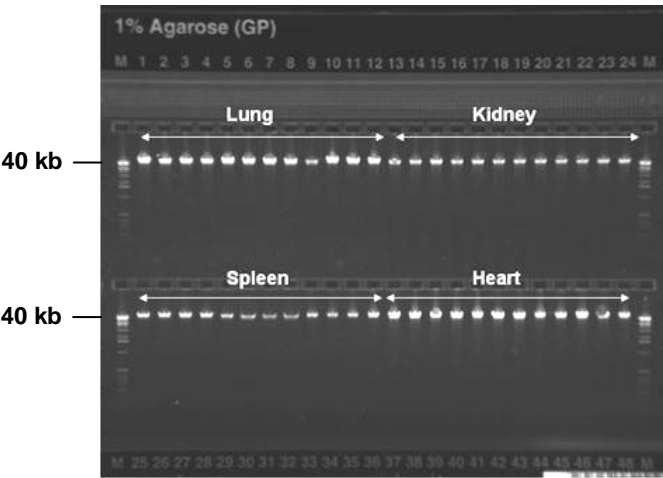
Lanes 1–12: Rat lung

Lanes 13–24: Rat kidney

Lanes 25–36: Rat spleen

Lanes 37–48: Rat heart

Lanes M: 1 Kb DNA Extension Ladder (0.5 µg/lane)



Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when purifying genomic DNA with the kit.

To troubleshoot problems with the iPrep™ Purification Instrument, refer to the manual supplied with the instrument.

Observation	Cause	Solution
Low DNA yield	Incomplete lysis	<ul style="list-style-type: none">• Decrease the amount of starting material used.• Be sure to add Proteinase K during lysis.• Make sure that the tissue is completely immersed in the Lysis Buffer.• Increase the length of incubation at 55°C.
	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 depends on the type and age of the starting material.
	Insufficient amount of ChargeSwitch® Magnetic Beads added	During shipping, some ChargeSwitch® Magnetic bead solution may adhere to the sealing foil of the cartridge. To collect any bead solution from the foil, tap the cartridge to deposit the bead solution at the bottom of the tube.

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Troubleshooting, Continued

Observation	Cause	Solution
No DNA recovered	ChargeSwitch [®] Magnetic Beads stored or handled improperly	<ul style="list-style-type: none"> • Store cartridge containing the beads at room temperature. Do not freeze the cartridge as the beads may be irreparably damaged. • Make sure that the beads are in solution at all times and do not dry. Dried beads are non-functional.
	iPrep [™] Tip blocked	Lysate contains particulate material that is blocking the iPrep [™] Tip. Remove any particulate material from the lysates prior to loading the samples onto the iPrep [™] Instrument.
Eluate containing DNA is discolored	Too much starting material used resulting in the presence of magnetic beads in the eluate	<p>Use the recommended amount of starting material.</p> <p>Remove any magnetic beads using a magnetic separator (MagnaRack[™] is available from Invitrogen, see page 19) or centrifuge the sample in a microcentrifuge for 1 minute at maximum speed.</p>
DNA is sheared or degraded	Lysate mixed too vigorously	Vortex gently to avoid DNA damage.
	Bubbles formed during mixing steps	Make sure that the sample volume is at least 600 μ L to prevent excessive bubble formation during mixing.
	Purified DNA repeatedly frozen and thawed	Aliquot purified DNA and store at 4°C (short-term) or -20°C (long-term). Avoid repeated freezing and thawing.
	DNA contaminated with DNases	Maintain a sterile environment while working (<i>i.e.</i> wear gloves and use DNase-free reagents).

Appendix

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the iPrep™ ChargeSwitch® gDNA Tissue Kit.

For more information about these products, visit www.invitrogen.com or call Technical Support (page 20).

Product	Amount	Cat. no.
iPrep™ Purification Instrument	1 unit	IS10000
iPrep™ Card: gDNA Tissue	1 card	IS10013
iPrep™ Card: Viral DNA/RNA	1 card	IS10016
iPrep™ Card: gDNA Blood	1 card	IS10012
iPrep™ Card: Buffy Coat	1 card	IS10015
iPrep™ Card: gDNA Forensic (includes buccal protocol)	1 card	IS10011
iPrep™ ChargeSwitch® Forensic Kit	1 kit (52 purifications)	IS10002
iPrep™ ChargeSwitch® Buccal Cell Kit	1 kit (52 purifications)	IS10003
iPrep™ ChargeSwitch® gDNA Tissue Kit	1 kit (52 purifications)	IS10004
iPrep™ PureLink™ Virus Kit	1 kit (52 purifications)	IS10008
iPrep™ Small Tips	1 bag of 52 tips	IS10111
iPrep™ Tip and Tube Rack	1 rack	IS10101
iPrep™ Cartridge Rack	1 rack	IS10102
Quant-iT™ PicoGreen® dsDNA Assay Kit	1 kit	P7589
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Qubit® Fluorometer	1 each	Q32857
MagnaRack™ Magnetic Separator	1 rack	CS15000

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. In addition, a large variety of DNA ladders is available from Invitrogen for sizing DNA. For more information about these products, see www.invitrogen.com or call Technical Support (page 20).

Technical Support

World Wide Web



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

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical support contact information
 - Access to the Invitrogen Online Catalog
 - Additional product information and special offers
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Contact Us

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

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Continued on next page

Technical Support, Continued

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