



# iPrep™ PureLink® gDNA Blood Kit

For purification of gDNA from human blood using the iPrep™ Purification Instrument

Catalog Number IS10005

Document Part Number 100001250 Publication Number MAN0000405 Revision 3 0



## Contents

Experienced Users Procedure	4
Kit Contents and Storage	6
Introduction	8
Product Overview	8
iPrep™ Purification Instrument	10
Methods	13
General Information	13
Isolating gDNA from Human Blood	15
DNA Quantitation and Analysis	19
Expected Results	20
Troubleshooting	21
Appendix	23
Accessory Products	23
Technical Support	24

## **Experienced Users Procedure**

#### Introduction

This quick reference sheet is included for experienced users of the  $iPrep^{TM}$  PureLink® gDNA Blood Kit. For more details, refer to this manual.

Step		Procedure
Purification Protocol	1.	Mix the fresh blood samples or thaw frozen blood samples or prepare buffy coat samples.
	2.	Open the iPrep <sup>™</sup> Card Slot and insert the iPrep <sup>™</sup> Card: gDNA Blood or iPrep <sup>™</sup> Card: Buffy Coat in the slot (arrow on the card is at the top and card label is facing your left side).
	3.	Turn $\mathbf{ON}$ the iPrep <sup>TM</sup> Instrument using the power switch on the left side of the instrument.
		The digital display shows the version for the iPrep™ which changes in few seconds to display the Main menu.
	4.	Press <b>Start</b> to run a protocol.
	5.	Open the $iPrep^{TM}$ instrument door and remove $iPrep^{TM}$ Racks to set up the platform.
	6.	Remove the iPrep <sup>™</sup> PureLink <sup>®</sup> gDNA Blood Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.
	7.	Insert one iPrep <sup>™</sup> Sample and Elution Tube <b>containing the sample</b> in the <b>heated tube position</b> of cartridge <b>(position 11)</b> for each of the iPrep <sup>™</sup> PureLink <sup>®</sup> gDNA Blood Cartridge that is used.
	8.	Load the cartridges on the $iPrep^{^{TM}}$ Cartridge Rack and insert the loaded rack on to the $iPrep^{^{TM}}$ Platform.

# Experienced Users Procedure, Continued

Step	Procedure			
Purification	9.	Load the iPrep <sup>™</sup> Tip and Tube Rack as follows:		
Protocol, Continued		a) Load the first row (labeled as <b>E</b> ) with 1–13 elution tubes <b>without caps</b> .		
		b) Keep the second row (labeled as T1) empty.		
		c) Load the third row (labeled as <b>T2</b> ) with iPrep <sup>™</sup> Tips in the iPrep <sup>™</sup> Tip Holders.		
		d) Keep the fourth row (labeled as S) empty.		
	10.	Read the sample and elution tube barcodes, if needed.		
	11.	Insert the $iPrep^{{\scriptscriptstyle TM}}$ Tip and Tube Rack on to the $iPrep^{{\scriptscriptstyle TM}}$ Platform.		
	12.	Close the iPrep $^{\text{\tiny TM}}$ instrument door. Press <b>Enter</b> ( $\  \  \  \  \  \  \  \  \  \  $ ) to continue.		
	13.	Select the appropriate elution volume on the display.		
	14.	Press <b>Start</b> . The automated purification protocol begins and various steps of the protocol including the approximate time remaining are displayed on the digital display.		
	15.	At the end of the run, the instrument beeps briefly and the digital display shows <b>Protocol Finished</b> for 10 seconds. The Main menu appears after 10 seconds.		
	16.	Open the instrument door.		
	17.	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).		
	18.	Discard the used cartridges, tips, and tubes into biohazard waste. <b>Do not</b> reuse the cartridges.		
	19.	To purify more samples using the same $iPrep^{TM}$ Card, load the racks with new cartridges, tips, tubes, and samples, and start the protocol as described.		
	20.	If you are not using the instrument, close the instrument door and turn the power switch to <b>OFF</b> .		
	21.	Remove the $iPrep^{TM}$ Card and store card in the box.		

### **Kit Contents and Storage**

# Shipping and Storage

The  $iPrep^{\mathbb{M}}$  PureLink® gDNA Blood Kit is shipped at room temperature.

Upon receipt, store the iPrep $^{\text{\tiny TM}}$  PureLink $^{\text{\tiny B}}$  gDNA Blood Kit at room temperature. See the following table for kit contents.

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

#### **Kit Contents**

The components supplied in the  $iPrep^{TM}$  PureLink® gDNA Blood Kit are listed below.

Sufficient reagents are supplied to perform 52 purifications.

Reagents	Amount
iPrep <sup>™</sup> PureLink <sup>®</sup> gDNA Blood Cartridge Kit	1 kit
iPrep <sup>™</sup> Sample and Elution Tubes	2 × 52 tubes
iPrep <sup>™</sup> Tips and Tip Holders	1 bag with 52 tips and holders

### Kit Contents and Storage, Continued

iPrep<sup>™</sup>
PureLink<sup>®</sup>
gDNA Blood
Cartridge Kit
Contents

Each iPrep<sup>TM</sup> PureLink<sup>®</sup> gDNA Blood 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 1–10 contain wells filled with reagents for this protocol.

The components supplied in each well of the  $iPrep^{TM}$  PureLink® gDNA Blood Cartridge Kit are listed in the following table.

Store the iPrep™ PureLink® gDNA Blood Cartridge Kit at room temperature and do not freeze the cartridge kit.

Reagent	Well No.
Lysis Buffer	1
Proteinase K (20 mg/mL) in storage buffer (proprietary)	2
Rinse Buffer	3
Dynabeads <sup>®</sup> MyOne <sup>™</sup> SILANE (4.8 mg/mL in Bead Storage Buffer)	4
100% Isopropanol	5
Wash Buffer 1	6
Wash Buffer 1	7
Wash Buffer 2	8
Wash Buffer 2	9
Elution Buffer	10

#### Introduction

#### **Product Overview**

#### Introduction

The iPrep™ PureLink® gDNA Blood Kit allows rapid and automated extraction of genomic DNA (gDNA) from human blood including fresh or frozen blood samples, and concentrated leukocyte preparations (buffy coat).

Genomic DNA is extracted from blood samples using the Dynabeads® MyOne™ SILANE and iPrep™ Purification Instrument within 30 minutes without the use of centrifugation.

The purified genomic DNA is suitable for use in downstream applications, including PCR.

# iPrep<sup>™</sup> Purification Instrument

The  $iPrep^{\mathbb{M}}$  PureLink® gDNA Blood Kit is designed for use with the  $iPrep^{\mathbb{M}}$  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 magnetic bead-based technology. See page 10 for details on the iPrep™ Purification Instrument.

#### System Overview

The  $iPrep^{\mathbb{M}}$  PureLink® gDNA Blood Kit combines the sensitivity and capacity of Dynabeads® MyOne<sup> $\mathbb{M}$ </sup> SILANE with the speed and convenience of the  $iPrep^{\mathbb{M}}$  Instrument to allow automated purification of high-quality DNA from up to 13 samples (12 samples + 1 positive control) within 30 minutes. The Dynabeads® MyOne<sup> $\mathbb{M}$ </sup> SILANE are monodisperse magnetic beads (1  $\mu$ m) with an optimized silica-like surface chemistry and a high specific surface area. Purification is achieved using a simple magnetic bead-based purification procedure, and avoids the use centrifuges or vacuum manifolds.

Cells are lysed using Lysis Buffer and proteins are digested with Proteinase K. The lysate is mixed with Dynabeads® MyOne™ SILANE 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.

### Product Product Overview, Continued

#### **Advantages**

The iPrep™ PureLink® gDNA Blood Kit provides the following advantages:

- Uses a magnetic bead-based technology to isolate genomic DNA without the need for centrifugation or vacuum manifolds
- Rapid and automated purification of genomic DNA within 30 minutes from a wide range of blood samples including difficult blood samples using the iPrep™ Instrument
- Pre-filled reagent cartridges provide easy set up and consistent results
- Minimal contamination with RNA
- Purified genomic DNA demonstrates improved downstream performance in applications such as PCR including multiplex PCR

# System Specifications

Starting Material: 350 µL human blood

300 µL buffy coat (see page 13)

Bead Size: ~1 µm
Bead Amount per Reaction: 2.4 mg
Number of Samples: Up to 13

Elution Volume: 100 μL, 150 μL, or 200 μL gDNA Yield:\* Up to 15 μg (whole blood)

Up to 48  $\mu$ g (1 × 10<sup>7</sup> concentrated

leukocyte prep)

DNA size: At least 40 kb

\*The gDNA yield depends on the sample volume and white blood cell count.

# iPrep<sup>™</sup> 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™ Protocol 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<sup>™</sup> Reaction Cartridge

The  $iPrep^{\mathbb{T}}$  Reaction Cartridges are supplied with  $iPrep^{\mathbb{T}}$  Kits and are designed to fit onto the  $iPrep^{\mathbb{T}}$  Cartridge Rack in only one orientation. Each cartridge is pre-filled with reagents required for the  $iPrep^{\mathbb{T}}$  PureLink® gDNA Blood 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™ PureLink® gDNA Blood Kit, positions 1–10 contain wells filled with reagents. You will insert a sample tube containing the blood sample in the heated tube position in the cartridge (position 11) as shown in the following image.

#### **Cartridge Specifications:**

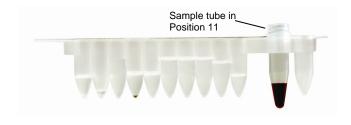
Material: Polypropylene cartridge sealed with

laminated aluminum foil

Max Volume: 1000 µL/well

Dimension:  $5.9 \text{ inches (l)} \times 1.2 \text{ inches (w)} \times 0.7 \text{ inches (d)}$ 

**Note:** The following image shows an example of an iPrep<sup>™</sup> PureLink<sup>®</sup> Blood Cartridge.



## iPrep<sup>™</sup> Purification Instrument, Continued

#### iPrep<sup>™</sup> Tips and Tip Holders

The iPrep™ Tips and Tip Holders are included with iPrep™ Kits and are placed on the iPrep™ Tip and Tube Rack as described on page 17. While assembling tips on the rack, insert the iPrep™ Tips into the iPrep™ Tip Holders using gloved hands. Always use tips with the holders to prevent any contamination.

#### **Tip Specifications:**

Tip Material: Polypropylene with filter barriers

Tip Holder Material: Polypropylene Volume: 5–1000 μL

Tip Dimension:  $3.9 \text{ inches (l)} \times 0.43 \text{ inches (d)}$ 

iPrep™ Tip Holder



iPrep™ Tip



### iPrep<sup>™</sup> Tubes

Two sets of iPrep<sup> $^{\text{IM}}$ </sup> Tubes are required for the purification protocol. The iPrep<sup> $^{\text{IM}}$ </sup> Sample and Elution Tubes are included with each iPrep<sup> $^{\text{IM}}$ </sup> Kit and placed on the iPrep<sup> $^{\text{IM}}$ </sup> Tip and Tube Rack as described on page 17.

#### **Tube Specifications:**

Material: Polypropylene

Capacity: 1.5 mL

Style: Tubes with caps

Dimensions:  $1.7 \text{ inches (l)} \times 0.4 \text{ inches (d)}$ 



## iPrep<sup>™</sup> Purification Instrument, Continued

#### iPrep<sup>™</sup> Card: gDNA Blood

To isolate gDNA from whole blood samples using the iPrep<sup>™</sup> PureLink<sup>®</sup> gDNA Blood Kit with the iPrep<sup>™</sup> Purification Instrument, you need to purchase the iPrep<sup>™</sup> Card: gDNA Blood (page 23).

The iPrep<sup>™</sup> Card: gDNA Blood is pre-programmed with the purification protocol for blood 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<sup>™</sup> Card: Buffy Coat

To isolate gDNA from concentrated leukocyte preparations (buffy coat) using the iPrep<sup>™</sup> PureLink<sup>®</sup> gDNA Blood Kit with the iPrep<sup>™</sup> Purification Instrument, you need to purchase the iPrep<sup>™</sup> Card: Buffy Coat (page 23).

The iPrep<sup>™</sup> Card: Buffy Coat is pre-programmed with the purification protocol for buffy coat that directs the volume of reagents used and incubation time.

**Note:** For samples containing less than  $2 \times 10^6$  leukocytes, use the iPrep<sup>TM</sup> Card: gDNA Blood protocol to achieve maximum yields.

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<sup>™</sup> Platform

The platform on the iPrep $^{\text{TM}}$  Instrument allows the placement of iPrep $^{\text{TM}}$  Tip and Tube Rack, and iPrep $^{\text{TM}}$  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 17 for the  $iPrep^{^{TM}}$  PureLink® gDNA Blood 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<sup>™</sup> Purification Instrument (page 23)
- iPrep<sup>™</sup> Card: gDNA Blood or iPrep<sup>™</sup> Card: Buffy Coat (page 23)
- Blood samples (see Blood Samples)

#### Blood Samples

The iPrep™ PureLink® gDNA Blood Kit is designed to purify high yield gDNA from various human blood samples including:

- Fresh, whole blood
- Blood collected in the presence of anti-coagulants such as EDTA or citrate
- Frozen blood samples or blood samples exposed to repeated freeze-thaw cycles
- Buffy coat (see **Preparing Buffy Coat**, page 14)

# Sample Volume

The iPrep<sup> $^{\text{TM}}$ </sup> PureLink<sup> $^{\text{®}}$ </sup> gDNA Protocol is designed to purify gDNA from up to 350  $\mu$ L human blood samples or up to 300  $\mu$ L buffy coat samples.

If sample volume is limited, you need to use at least 150  $\mu L$  sample volume. Do not use less than 150  $\mu L$  blood sample volume as using less sample volume results in excessive bubble formation during the purification protocol thereby lowering the gDNA yield.

#### Safety Information

Follow the safety guidelines below when using the iPrep<sup>™</sup> PureLink® gDNA Blood Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles when handling whole blood samples.
- Dispose of blood samples as biohazardous waste.

### General Information, Continued

# Preparing Buffy Coat

Use the following protocol to prepare buffy coat sample. Leukocyte concentration can be enriched by up to 10-fold using this method.

- 1. Centrifuge whole blood sample at  $300 \times g$  for 10 minutes at room temperature.
- Following centrifugation, the erythrocytes (RBC) pellet to
  the bottom of the tube while the plasma migrates to the
  top of the tube. There is a thin layer at the interface of the
  plasma and red cells that contains concentrated
  leukocytes and platelets. This thin layer is the buffy coat
  layer.
- 3. Using a sterile pipette, remove the plasma layer to within 1–2 mm of the interface.
- 4. Using fresh, sterile pipette, remove the buffy coat layer containing the remaining plasma, leukocyte (buffy coat) interface, and approximately 1–2 mm of the red blood cell layer to a sterile microcentrifuge tube.
- 5. Use the concentrated leukocyte preparation (buffy coat) for gDNA isolation or store buffy coat at 4°C for 1 week, or at -20°C or -80°C for long-term storage.



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 freeze the beads as this irreparably damages them. Store the beads at room temperature.
- When using 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<sup>™</sup> Tips, and iPrep<sup>™</sup> Tip Holders after use. Do not reuse.

### **Isolating gDNA from Human Blood**

#### Introduction

Instructions to isolate genomic DNA from human blood samples using the iPrep™ PureLink® gDNA Blood Kit with the iPrep™ Purification Instrument are described below.

#### Starting Material

Use this procedure to isolate genomic DNA from up to  $350~\mu L$  human blood samples or up to  $300~\mu L$  concentrated leukocyte preparations (buffy coat). See page 13 for sample volume.

#### Materials Needed

Components Supplied by the user

- Blood samples (see page 13)
- iPrep<sup>™</sup> Purification Instrument (page 23)
- iPrep<sup>™</sup> Card: gDNA Blood or iPrep<sup>™</sup> Card: Buffy Coat (page 23)

Components Supplied with the Kit

- iPrep<sup>™</sup> PureLink<sup>®</sup> gDNA Blood Cartridge Kit
- iPrep<sup>™</sup> Sample and Elution Tubes
- iPrep<sup>™</sup> Tips and iPrep<sup>™</sup> Tip Holders

# Before Starting

Perform the following before starting:

- Thaw frozen blood samples or mix the fresh blood samples or prepare buffy coat samples, and store on ice until use
- Ensure that you have the iPrep<sup>™</sup> Card: gDNA Blood or iPrep<sup>™</sup> Card: Buffy Coat (page 23) to run the protocol
- Make sure the iPrep<sup>™</sup> Purification Instrument is unpacked and installed

### Isolating gDNA from Human Blood, Continued

# Purification Protocol

Purify genomic DNA from human blood samples using the iPrep<sup>™</sup> Purification Instrument as described.

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

Insert the iPrep<sup>™</sup> Card: gDNA Blood or iPrep<sup>™</sup> Card: Buffy Coat (available separately from Life Technologies, page 23) prior to turning on the instrument.

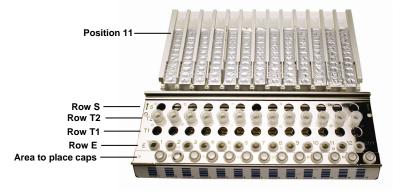
- 1. Ensure the power switch on the iPrep<sup>™</sup> Instrument is on the **OFF** position.
- 2. Open the iPrep<sup>™</sup> Card Slot and insert the iPrep<sup>™</sup> Card into the slot in the correct orientation (arrow on the card is at the top and card label is facing your left side).
- 3. Using the 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  $iPrep^{\text{\tiny M}}$  which changes in few seconds to display the Main menu.
- 4. Press **Start** to run a protocol.
- 5. Open the iPrep<sup>™</sup> instrument door. Remove the iPrep<sup>™</sup> Cartridge Rack, and iPrep<sup>™</sup> Tip and Tube Rack to set up the platform.
- Remove the desired number of iPrep<sup>™</sup> PureLink<sup>®</sup> gDNA Blood Cartridges from the box. To collect any solution from the foil, tap the cartridge to deposit the solution at the bottom of the tube.

**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.

### Isolating gDNA from Human Blood, Continued

# Purification Protocol, Continued

- 7. Add the blood sample to an iPrep<sup>™</sup> Sample and Elution Tube. Insert the iPrep<sup>™</sup> Sample and Elution Tube with sample in the heated tube position of the cartridge (position 11) for each of the iPrep<sup>™</sup> gDNA Blood Cartridge that is used.
- 8. Load the cartridges on the iPrep<sup>™</sup> Cartridge Rack and insert the loaded rack on the iPrep<sup>™</sup> platform.
- 9. Load the iPrep<sup>™</sup> Tip and Tube Rack as follows (see the following figure):
  - 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 row (labeled as T1) empty
  - Load the third row (labeled as T2) with iPrep<sup>™</sup> Tips in the iPrep<sup>™</sup> Tip Holders
  - Keep the fourth row (labeled as S) empty



- 10. Read the sample and elution tube barcode, if needed.
- 11. Insert the iPrep Tip and Tube rack on the iPrep<sup>™</sup> platform as shown above.
- 12. Close the iPrep<sup>™</sup> instrument door.

### Isolating gDNA from Human Blood, Continued

# Purification Protocol, Continued

- 13. Press **Enter**  $(\ \ )$  to continue.
- 14. Select the appropriate elution volume on the display, when prompted.
- 15. Ensure that you have loaded the cartridges, tubes, and tips in the appropriate positions, and elution tubes do not have any caps. Make sure you have loaded a tube with sample in the heated tube position of the cartridge (position 11).
- 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  $iPrep^{\mathbb{M}}$  Instrument manual.

- 17. 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.
- 18. Open the instrument door. Remove and cap the elution tubes containing the purified nucleic acid. Store the purified gDNA as described (see **Storing DNA**).
- 19. Discard the used cartridges, tips, and sample tubes into biohazard waste. **Do not reuse the cartridges.**
- 20. To purify more samples using the same iPrep<sup>™</sup> Card, load the racks with new cartridges, tips, and samples, and start the protocol as described (steps 6–19).
- 21. If you are not using the instrument, close the instrument door and turn the power switch to **OFF**.
- 22. Remove the iPrep<sup>™</sup> Card and store the card in the box, protected from light.

#### Storing DNA

- Use the purified DNA immediately for the desired downstream application.
- Aliquot purified DNA and store at 4°C (short-term) or -20°C (long-term). Avoid repeated freezing and thawing.

### **DNA Quantitation and Analysis**

#### **DNA Yield**

Perform DNA quantitation using UV absorbance at 260 nm or Quant- $iT^{\text{TM}}$  Kits.

#### **UV** Absorbance

- 1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance at 260 nm (A<sub>260</sub>) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5.
- 2. Calculate the concentration of DNA using the formula: DNA ( $\mu g/mL$ ) =  $A_{260} \times 50 \times$  dilution factor For DNA,  $A_{260} = 1$  for a 50  $\mu g/mL$  solution measured in a cuvette with an optical path length of 1 cm.

#### Quant-iT<sup>™</sup> Kits

The Quant-iT™ Kits (see page 23 for ordering information) provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. The kit contains a state-of-the-art quantitation reagent, DNA standards for standard curve, and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers/fluorometers or the Qubit® 2.0 Fluorometer.

# Analyzing DNA Quality

Typically, DNA isolated using the iPrep<sup>™</sup> PureLink<sup>®</sup> gDNA Blood Kit has an  $A_{260}/A_{280} > 1.70$  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.

### **Expected Results**

# Expected Yield and Purity

Examples of expected yield and purity are given as mean values, with the range of values in parentheses.

DNA was purified using the iPrep<sup>™</sup> PureLink<sup>®</sup> gDNA Blood Kit and the iPrep<sup>™</sup> Purification Instrument using five different blood samples. DNA was eluted in 200  $\mu$ L elution buffer; yield was determined using the Quant-iT<sup>™</sup> Pico-Green<sup>®</sup> dsDNA Assay. The UV absorbance ratios were measured using a NanoDrop<sup>®</sup> ND-1000 spectrophotometer.

Sample (treatment)	DNA yield (range)	Purity A <sub>260</sub> /A <sub>280</sub> (range)	Purity A <sub>260</sub> /A <sub>230</sub> (range)
Fresh blood	6.0 μg	1.81	1.85
(EDTA)	(5.2–7.5)	(1.80–1.83)	(1.75–1.91)
Frozen blood	10.0 µg	1.82	1.83
(EDTA)	(7.7–13.0)	(1.78–1.88)	(1.72– 1.91)
Frozen blood	8.7 μg	1.83	1.91
(citrate)	(6.7–12.1)	(1.77–1.89)	(1.75–2.05)
Buffy coat (300 µL input material)	23.1 (4.6–47.1)	1.84 (1.75–1.90)	2.12 (1.80–2.35)

## **Troubleshooting**

#### Introduction

Refer to the table below to troubleshoot problems with the kit. To troubleshoot problems with the  $iPrep^{TM}$  Purification Instrument, refer to the instrument manual.

Observation	Cause	Solution
Low DNA yield	Too much starting material used	The purification protocol is designed for use with 350 $\mu$ L blood volume or 300 $\mu$ L buffy coat (maximum of 1 × 10 <sup>7</sup> leukocytes). Using greater than the recommended amount of starting material may overload the system and cause clumping which reduces the yield.
	Insufficient amount of Dynabeads® MyOne™ SILANE added	During shipping, some Dynabeads® MyOne™ SILANE 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.
	Used less sample volume	The protocol is designed for use with 350 $\mu$ L human blood sample or 300 $\mu$ L buffy coat. You may lower the sample volume to 150 $\mu$ L but using <150 $\mu$ L sample volume produces bubbles during the purification protocol resulting in low DNA yield.
	Low leukocyte count in buffy coat preparation	Use iPrep <sup>™</sup> Card: gDNA Blood protocol if anticipated yield is lower than 15 µg or the leukocyte count is less than 2 × 10 <sup>6</sup> cells.
No DNA recovered	Magnetic beads stored or handled	Store cartridge containing the beads at room temperature. Do not freeze the cartridge as the beads may be irreparably damaged.
	improperly	Make sure that the beads are in solution at all times and do not dry. Dried beads are non-functional.
DNA eluate is discolored	Magnetic beads present in the eluate	Remove any magnetic beads using a magnetic separator (MagnaRack™ separator is available from Life Technologies, see page 23) or centrifuge the sample in a microcentrifuge for 1 minute at maximum speed.

# Troubleshooting, Continued

Observation	Cause	Solution
DNA is sheared or degraded	Bubbles formed during mixing steps	Make sure that the sample volume is at least $150\mu\text{L}$ 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.e., wear gloves and use DNase-free reagents).

### **Appendix**

### **Accessory Products**

# Additional Products

The following table lists additional products available from Life Technologies that may be used with the iPrep<sup>TM</sup> PureLink® gDNA Blood Kit. For more information, visit **www.lifetechnologies.com** or contact Technical Support (page 24).

Product	Amount	Cat. No.
iPrep <sup>™</sup> Purification Instrument	1 unit	IS10000
iPrep™ Card: gDNA Blood	1 card	IS10012
iPrep <sup>™</sup> Card: Buffy Coat	1 card	IS10015
iPrep <sup>™</sup> Card: gDNA Tissue	1 card	IS10013
iPrep <sup>™</sup> Card: gDNA Forensic (includes buccal protocol)	1 card	IS10011
iPrep <sup>™</sup> Card: Viral DNA/RNA	1 card	IS10016
iPrep <sup>™</sup> ChargeSwitch® Forensic Kit	1 kit (52 purifications)	IS10002
iPrep <sup>™</sup> ChargeSwitch® Buccal Cell Kit	1 kit (52 purifications)	IS10003
iPrep <sup>™</sup> ChargeSwitch® gDNA Tissue Kit	1 kit (52 purifications)	IS10004
iPrep™ PureLink® Virus Kit	1 kit (52 purifications)	IS10008
iPrep <sup>™</sup> 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 <sup>™</sup> DNA Assay Kit, Broad-Range	1000 assays	Q33130
Qubit® 2.0 Fluorometer	1 each	Q32866
MagnaRack <sup>™</sup> Magnetic Separator	1 rack	CS15000

# E-Gel<sup>®</sup> 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 Life Technologies for sizing DNA. For more information call Technical Support (page 24) or visit www.lifetechnologies.com.

### **Technical Support**

#### Obtaining Support

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

At the website, you can:

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

#### Safety Data Sheets (SDS)

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

#### Certificate of Analysis

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

### **Technical Support, Continued**

#### Limited Product Warranty

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

©2012 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. NanoDrop is a registered trademark of NanoDrop Technologies, LLC.

#### **Disclaimer**

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

### **Notes**

#### Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA Phone +1 760 603 7200 | Toll Free in USA 800 955 6288 For support visit

 $\textbf{lifetechnologies.com/support} \ \textbf{or} \ \textbf{email} \ \textbf{techsupport} \\ \textbf{@lifetech.com}$ 

lifetechnologies.com

