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# EZ1&2™ DNA Blood Handbook

For automated purification of DNA from blood and buffy coat using EZ2® Connect instruments

# Contents

Kit Contents .....	3
Shipping and Storage .....	4
Intended Use .....	4
Safety Information .....	5
Quality Control .....	6
Introduction .....	7
Principle and procedure .....	7
Equipment and Reagents to Be Supplied by User .....	8
Important Notes .....	9
Starting material .....	9
Precipitate in reagent cartridge .....	10
Quantification of DNA .....	11
Working with EZ2 Connect instruments .....	11
Yield of purified DNA .....	15
Protocol: Purification of DNA from Whole Blood .....	16
Protocol: Purification of DNA from Buffy Coat .....	19
Troubleshooting Guide .....	22
Appendix A: Storage, Quantification and Determination of Purity of DNA .....	25
Ordering Information .....	27
Document Revision History .....	28

# Kit Contents

<b>EZ1&amp;2 DNA Blood Kits</b>	<b>200 µl</b>	<b>350 µl</b>
<b>Catalog no.</b>	<b>951034</b>	<b>951054</b>
<b>No. of preps</b>	<b>48</b>	<b>48</b>
Reagent Cartridge, Blood 200 µl (cat. no. 1023745)	48	–
Reagent Cartridge, Blood 350 µl (cat. no. 1023729)	–	48
Disposable Tip Holders	50	50
Disposable Filter-Tips	50	50
Sample Tubes (2 ml)	50	50
Elution Tubes (1.5 ml)	50	50
Q-Card*	1	1
Quick-Start Protocol	1	1

\* The information encoded in the bar code on the Q-Card is needed for reagent data tracking using EZ2 Connect instruments.

# Shipping and Storage

The EZ1&2 DNA Blood Kits are shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent cartridges. When stored properly, the reagent cartridges are stable until the expiration date on the Q-Card.

## Intended Use


The EZ1&2 DNA Blood 200 µl Kit and the EZ1&2 DNA Blood 350 µl Kit are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

The EZ1&2 DNA Blood Kits are intended to be used with EZ1 instruments and EZ2 Connect instruments from QIAGEN.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety), where you can find, view and print the SDS for each QIAGEN kit and kit component.

<p><b>CAUTION</b></p> 	<p>DO NOT add bleach or acidic solutions directly to the sample-preparation waste.</p>
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Buffers in the reagent cartridges contain guanidine hydrochloride or guanidine thiocyanate, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite. If liquid containing potentially infectious agents is spilt on the EZ2 Connect instrument, clean the affected area first with laboratory detergent and water, and then with disinfectants and detergents compatible with metallic surfaces as listed in the *EZ2 Connect and EZ2 Connect Fx User Manual*.

# Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the EZ1&2 DNA Blood Kits is tested against predetermined specifications to ensure consistent product quality.

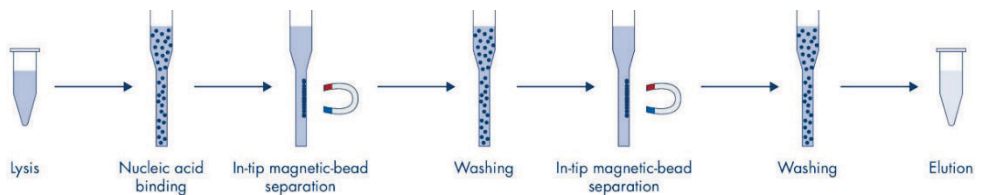
# Introduction

The EZ1&2 DNA Blood 200 µl Kit and the EZ1&2 DNA Blood 350 µl Kit are for purification of genomic DNA from whole blood samples on the EZ2 Connect. In addition, this handbook also describes processing buffy coat using the EZ1&2 DNA Blood 350 µl Kit on the EZ2 Connect. To use the EZ1&2 DNA Blood 200 µl Kit and the EZ1&2 DNA Blood 350 µl Kit with EZ1 instruments, refer to the corresponding handbook ([www.qiagen.com/HB-0197](http://www.qiagen.com/HB-0197)) and quick-start protocol ([www.qiagen.com/HB-0782](http://www.qiagen.com/HB-0782)).

Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications, such as amplification or other enzymatic reactions. The EZ2 Connect instruments perform all steps of the sample preparation procedure, and the procedure can be scaled up or down, allowing purification from varying amounts of starting material. Up to 24 samples can be processed in a single run.

## Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles (Figure 1). DNA is isolated from lysates in one step by binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted with elution buffer.



**Figure 1. EZ1&2 DNA extraction procedure.**

# Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

## All protocols

- Soft paper tissue
- Water
- 70% ethanol
- Pipettes
- Thermomixer, heated orbital incubator, heating block or water bath

## For blood

- **Optional:** 80% ethanol and 2 ml screw-capped tubes (if the 80% ethanol wash step is performed, see “Important points before starting”, page 16).

## For buffy coat

- 1.5 ml or 2 ml screw-capped tube



# Important Notes

## Starting material

The amounts of starting material for use in EZ1&2 DNA Blood procedures are shown in Table 1.

**Table 1. Amounts of starting material for EZ1&2 DNA Blood procedures**

Sample type	Amount of starting material (µl)	Elution volume (µl)
Blood	200 or 350	50, 100, or 200
Buffy coat*		
Buffy coat, enriched >9x <sup>†</sup>	50–75	200
Buffy coat, enriched 9x <sup>‡</sup>	100–150	200
Buffy coat with low leukocyte concentration <sup>§</sup>	200–300	200

\* For each buffy coat protocol, use a maximum of  $5 \times 10^6$  cells as starting material.

<sup>†</sup> For example, 1 ml leukocyte containing the fraction harvested from 10 ml centrifuged whole blood = 10x enrichment.

<sup>‡</sup> This is recommended for preparation of buffy coat.

<sup>§</sup> For example, from certain leukemia patients or other donors where leukocyte count is low.

## Storage of blood

Whole blood samples treated with EDTA, ACD or heparin\* can be used and may be either fresh or frozen. In general, follow instructions and guidelines of the blood collection tube provider.

\* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Frozen samples should be thawed at room temperature (15–25°C) with mild agitation before beginning the procedure. Yield and quality of the purified DNA depend on storage conditions of the blood. Fresher blood samples may yield better results.

- For short-term storage (up to 10 days), collect blood in tubes containing EDTA as an anticoagulant, and store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, because low levels of DNA degradation will occur after this time.
- For long-term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store the tubes at –70 to –80°C.

## Buffy coat

Buffy coat may vary considerably in leukocyte concentration, depending upon the number of nucleated cells in the original whole blood sample and the efficiency of leukocyte harvesting during the buffy coat preparation. To avoid overloading the isolation procedure when using highly enriched buffy coat samples (>9x enrichment), smaller volumes of starting material should be used (see Table 1, page 9, for recommended starting volumes). Efficiency of buffy-coat enrichment depends on the sample preparation procedure used and on the accuracy used when extracting the buffy coat layer. Three different protocols are available for purification of genomic DNA from buffy coat on the EZ2 Connect instrument. The amounts of recommended starting material for the three different protocols are shown in Table 1.

## Precipitate in reagent cartridge

The buffer in well 1 of the reagent cartridge (see Figure 2) may form a precipitate upon storage. If necessary, redissolve by mild agitation at 37°C.

## Quantification of DNA

Carryover of magnetic particles may affect the absorbance reading at 260 nm ( $A_{260}$ ) of the purified DNA but should not affect downstream applications. The measured absorbance at 320 nm ( $A_{320}$ ) should be subtracted from all absorbance readings. See “Quantification of DNA” in Appendix A (page 25), for more information.

## Working with EZ2 Connect instruments

The main features of EZ2 Connect instruments include:

- Small footprint to save laboratory space
- Preprogrammed protocols for nucleic acid purification
- Prefilled, sealed reagent cartridges for easy, safe and fast run setup
- Complete automation of nucleic acid purification, from opening of reagent cartridges to elution of nucleic acids, with no manual centrifugation steps
- Optional bar code reading and sample tracking
- Kit data tracking with the Q-Card provided in the kit
- UV LED to help eliminate sample carryover from run-to-run and to allow pathogen decontamination on the worktable surfaces

**Note:** UV decontamination helps to reduce possible pathogen contamination of the EZ2 Connect worktable surfaces. The efficiency of inactivation has to be determined for each specific organism and depends, for instance, on layer thickness and sample type. QIAGEN cannot guarantee complete eradication of specific pathogens.

## Reagent cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 2). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer or elution buffer. Because each well contains only

the required amount of reagent, generation of waste due to leftover reagent at the end of the purification procedure is avoided.



**Figure 2. Ease of worktable setup using reagent cartridges. (A)** A sealed, prefilled reagent cartridge. Fill levels vary, depending on the type of reagent cartridge. **(B)** Loading reagent cartridges into the cartridge rack. The cartridge rack itself is labeled with an arrow to indicate the direction in which reagent cartridges must be loaded. Each reagent cartridge contains 12 individual positions.

## EZ2 Connect tip racks

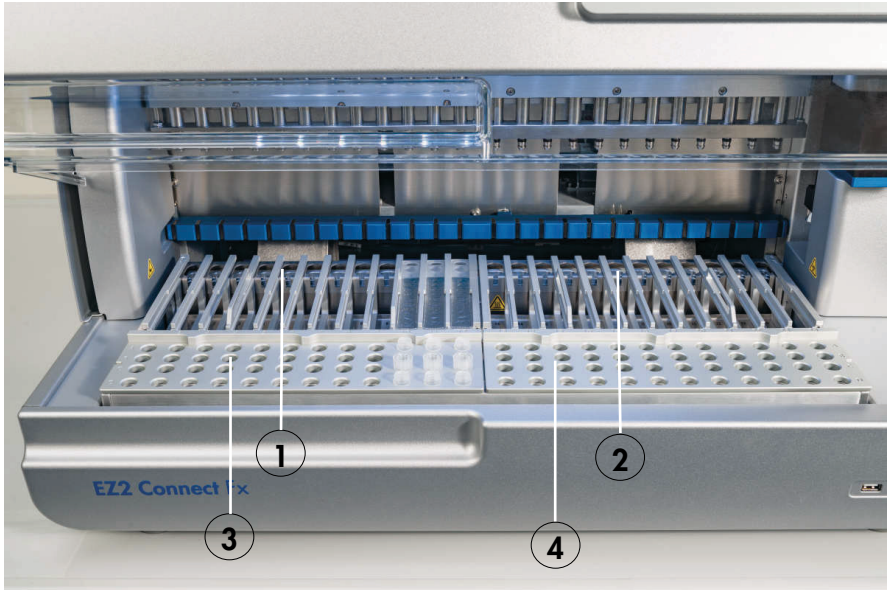
The EZ2 Connect tip racks holds tips inserted into tip holders and tubes for samples or elution. Details on how to load the tip racks are displayed during the run setup on the LED display of the EZ2 Connect.



**Figure 3. The EZ2 Connect Tip Rack. (A)** Each Tip Rack has 4 positions, which are labeled A–D. **(B)** The rack is designed to hold sample and elution tubes as well as tips in their corresponding tip holders.

## Worktable

The worktable of EZ2 Connect instruments is where the user loads samples and the components of the EZ1&2 Kits (Figure 4).



**Figure 4. EZ2 Connect Worktable.**

- |                                      |                                       |
|--------------------------------------|---------------------------------------|
| 1. EZ2 Connect Cartridge Rack – left | 2. EZ2 Connect Cartridge Rack – right |
| 3. EZ2 Connect Tip Rack – left       | 4. EZ2 Connect Tip Rack – right       |

## Operation of the EZ2 Connect

The EZ2 Connect provides various features to support the sample preparation workflow. These include functions for remote access via QIA®sphere, data input via bar code reading, data storage and transfer, report generation and guided instrument maintenance. For more information about these features, refer to the *EZ2 Connect and EZ2 Connect Fx User Manual*.

## Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample and the protocol used for purification of DNA. Table 2 shows typical yields obtained from different sample volumes and sample types.

**Table 2. DNA yields obtained from different sample types using EZ1&2 DNA procedures**

<b>Sample type</b>	<b>Sample amount</b>	<b>DNA yield</b>
Blood*	200 µl	4–8 µg
Blood*	350 µl	5–12 µg
Buffy coat enriched >9x†	75 µl	6.5–14.5 µg
Buffy coat enriched 9x	150 µl	8–14 µg
Buffy coat with low leukocyte concentration	300 µl	Up to 14 µg

\* Whole blood with  $4\text{--}7 \times 10^6$  white blood cells/ml; elution volume 200 µl.

† Prepared from blood bag, 10x enrichment. This type of buffy coat preparation tends to result in very efficient leukocyte enrichment.

# Protocol: Purification of DNA from Whole Blood

## Important points before starting

- If using the EZ1&2 DNA Blood 200 µl Kit or the EZ1&2 DNA Blood 350 µl Kit for the first time, read “Important Notes”, page 9.
- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (page 3). Do not use damaged kit components, because their use may lead to poor kit performance.
- The protocols include an option to perform 80% ethanol washes instead of washes using the buffer provided in the reagent cartridge. This may be advantageous for some downstream applications. If this option is selected, 2 ml tubes containing 1800 µl 80% ethanol should be placed in row 3 of the worktable by the user (see Figure 4, page 14). Follow the instructions given in the on-screen messages.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 3 for “Safety Information”.
- All steps of the protocol should be performed at room temperature (15–25°C). During the setup procedure, work quickly.



## Things to do before starting

- The buffer in well 1 of the reagent cartridge (Figure 2) may form a precipitate upon storage. If necessary, redissolve by warming at 37°C and then place at room temperature (15–25°C).
- **Important:** Do not shake the cartridge vigorously, because shaking too hard will lead to foaming of the buffer.
- Equilibrate reagent cartridges to room temperature before use.
- If using fresh blood, mix the blood samples thoroughly before loading them onto the EZ2 Connect instrument to ensure homogeneity of the sample.
- If using frozen blood samples, thaw the blood samples and equilibrate to room temperature. Mix the blood samples thoroughly before loading them onto the EZ2 Connect instrument to ensure homogeneity of the sample.

## Procedure

1. Switch on the EZ2 Connect instrument.
2. Tap “DNA” on the Applications panel and then select the “DNA Blood 200 µl Kit” or the “DNA Blood 350 µl Kit” and press **Next**.
3. Choose the “DNA Blood” protocol and press **Next**.
4. Choose sample and elution volume. If the wash step should be performed with ethanol, select the corresponding option and press **Next**.
5. Select positions on the work deck according to the number of samples to be processed and press **Next**.
6. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
7. Gently invert reagent cartridges four times to mix the magnetic particles. Tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

8. Load the EZ1&2 Blood reagent cartridges into the corresponding positions of the EZ2 Connect Cartridge Rack as selected in step 5.
9. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
10. Remove caps of from all tubes, and prepare the EZ2 Connect Tip Rack as follows (Figure 3):
  - Position A: 2.0 ml sample tube
  - Position B: 2.0 ml with 1800  $\mu$ l 80% ethanol (optional, see step 4)
  - Position C: Tip holder with Filter Tip
  - Position D: 1.5 ml empty elution tube
  - Press **Next**.
11. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
12. The display will show "Protocol finished" when the run is completed. Press **Finish**.
13. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position D of the EZ2 Connect Tip Rack. Discard the used cartridge, including the liquid waste.

**Optional:** Follow on-screen instructions for UV decontamination of worktable surfaces.
14. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

**Optional:** Follow on-screen instructions for UV decontamination of worktable surfaces.

# Protocol: Purification of DNA from Buffy Coat

## Important points before starting

- If using the EZ1&2 DNA Blood Kit or working with buffy coat for the first time, read “Important Notes”, page 9.
- After receiving the kit, check the kit components for damage. If any kit components are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (page 5). Do not use damaged kit components, since their use may lead to poor kit performance.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. Take appropriate safety measures and wear gloves when handling. See page 5 for “Safety Information”.
- All steps of the protocol should be performed at room temperature. During the setup procedure, work quickly.

## Things to do before starting

- The buffer in well 1 of the reagent cartridge (Figure 2) may form a precipitate upon storage. If necessary, redissolve by warming at 37°C and then place at room temperature (15–25°C).  
**Important:** Do not vigorously shake the cartridge, as it will lead to foaming of the buffer.
- Equilibrate reagent cartridges to room temperature before use.
- If using fresh buffy coat, mix the buffy coat samples thoroughly before loading them onto the EZ2 Connect instrument to ensure homogeneity of the sample.
- If using frozen buffy coat samples, thaw the buffy coat samples and equilibrate to room temperature. Mix the buffy coat samples thoroughly before loading them onto the EZ2 Connect instrument to ensure homogeneity of the sample.

## Procedure

### Preparation of buffy coat

1. Centrifuge whole blood at  $300 \times g$  for 10 min at room temperature (15–25°C).

Whole blood samples containing a standard anticoagulant (EDTA, citrate or heparin) should be used.

After centrifugation, three different layers are visible: the clear upper layer is plasma, the intermediate layer is buffy coat containing concentrated leukocytes and the bottom layer contains concentrated erythrocytes.

2. Carefully transfer the middle layer containing the concentrated leukocytes to a new tube (not supplied). First, pipet as much of the gray-white interface as possible, followed by equal portions of the layers directly above and below the interface.

In some cases, it may be helpful to carefully aspirate part of the plasma layer before harvesting the leukocytes.

A 1.8 ml whole blood sample should yield approximately 200  $\mu$ l buffy coat. Scaling up the preparation (e.g., to obtain 1 ml buffy from 9 ml whole blood) may improve the efficiency of the leukocyte harvest.

3. Proceed with DNA purification immediately, or store samples at –30 to –15°C for purification at a later date.

### DNA purification

1. Switch on the EZ2 Connect instrument.
2. Tap “DNA” on the Applications panel and then select the “DNA Blood 350  $\mu$ l Kit” and press **Next**.
3. Choose the “DNA Buffy Coat” protocol and press **Next**.
4. Choose sample volume and press **Next**.

5. Select positions on the work deck according to the number of samples to be processed and press **Next**.
6. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.
7. Gently invert reagent cartridges four times to mix the magnetic particles. Tap the cartridges to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.
8. Load the EZ1&2 Blood reagent cartridges into the corresponding positions of the EZ2 Connect Cartridge Rack as selected in step 5.
9. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument.
10. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows (Figure 3):
  - Position A: 2.0 ml sample tube
  - Position B: empty
  - Position C: Tip holder with Filter Tip
  - Position D: 1.5 ml empty elution tube
  - Press **Next**.
11. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
12. The display will show "Protocol finished" when the run is completed. Press **Finish**.
13. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position D of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

**Optional:** Follow on-screen instructions for UV decontamination of worktable surfaces.
14. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

**Optional:** Follow on-screen instructions for UV decontamination of worktable surfaces.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page in our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook (for contact information, visit [support.qiagen.com](http://support.qiagen.com)).

## Comments and suggestions

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### General handling

- a) Error message in instrument display      Refer to the user manual supplied with your EZ2 Connect instrument.

### Low DNA yield or dirty filter tips

- a) Magnetic particles not completely resuspended      Ensure that you invert the reagent cartridges several times to resuspend the magnetic particles.
- b) Insufficient reagent aspirated      After inverting the reagent cartridges to resuspend the magnetic particles, ensure that you tap the cartridges to deposit the reagents at the bottom of the wells.
- c) Varying pipetting volumes      To ensure pipetting accuracy, ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date, as the latter can result in the loss of fluids. Do not use cartridges with visible foam.  
Also, perform regular maintenance as described in the instrument user manual, because greasing the O-rings of the dilutors affects the fit of the filter tips.
- d) Frozen blood or buffy coat samples not mixed properly after thawing      Thaw frozen blood or buffy coat samples at room temperature (15–25°C), with mild agitation to ensure thorough mixing.

## Comments and suggestions

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- e) Blood sample is clotted
- Ensure that the tubes used to collect and store whole blood contain EDTA, ACD or heparin as anticoagulant. Follow the instructions of the collection tube provider.
- Fresh blood can be stored at 2–8°C for up to 10 days.
- Frozen blood should be in tubes containing EDTA. It can keep for more than 10 days if stored at –70 to –80°C.
- f) Beads transgressed the filter
- Dilutor may be impaired. Contact field service through [support.qiagen.com](mailto:support.qiagen.com).

## DNA does not perform well in downstream applications

- a) Insufficient DNA used in downstream application
- Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of DNA”, Appendix A, page 25).
- b) Excess DNA used in downstream application
- Excess DNA can inhibit some enzymatic reactions. Quantify the purified DNA by spectrophotometric measurement of the absorbance at 260 nm (see “Quantification of DNA”, Appendix A, page 25).
- c) Inhibition of downstream application
- Downstream applications may show superior performance if an 80% ethanol wash is performed instead of washes using buffers in the reagent cartridges (see page 16).
- d) Poor storage conditions led to poor-quality DNA
- For storage of up to 10 days, collect blood in tubes with EDTA as anticoagulant, and store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, we recommend storage at 2–8°C for up to 3 days only, because low levels of DNA degradation will occur after this time.
- For storage longer than 10 days, collect blood in tubes containing a standard anticoagulant (preferably EDTA, if high-molecular-weight DNA is required), and store the tubes at –70 to –80°C.
- Do not use blood that shows signs of coagulation. Fresher blood samples may yield better results. Follow the collection tube provider’s instructions.

## Low $A_{260}/A_{280}$ ratio for purified nucleic acids

Absorbance reading at 320 nm not subtracted from the absorbance readings obtained at 260 nm and 280 nm

To correct for the presence of magnetic particles in the eluate, an absorbance reading at 320 nm should be taken and subtracted from the absorbance readings obtained at 260 nm and 280 nm (see “Quantification of DNA”, Appendix A, page 25).

## Comments and suggestions

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### Low DNA yield from buffy coat

- |  |   |
|--|---|
| a) Clogging due to sample overload               | Reduce the amount of sample. The maximum recommended amount of cells to use as starting material is $5 \times 10^6$ . |
| b) Poor buffy coat preparation                   | Ensure that the leukocyte fraction is harvested efficiently.  |
| c) Low leukocyte count in the whole blood sample | Increase whole blood amount and keep the volume of leukocytes harvested constant.                                     |



# Appendix A: Storage, Quantification and Determination of Purity of DNA

## Storage of DNA

Purified DNA may be stored at 2–8°C for 24 h or at –30 to –15°C for longer storage.

## Quantification of DNA

The concentration of DNA should be determined by measuring the absorbance at 260 nm ( $A_{260}$ ) in a spectrophotometer. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. An absorbance of 1 unit at 260 nm corresponds to 50 µg of DNA per ml ( $A_{260} = 1 \rightarrow 50 \mu\text{g/ml}$ ). Use buffer of neutral pH (e.g., 10 mM Tris-Cl, \* pH 7.0) to dilute the samples and to calibrate the spectrophotometer.† Carryover of magnetic particles in the eluate may affect the  $A_{260}$  reading but should not affect the performance of the DNA in downstream applications. If the purified DNA is to be analyzed by fluorescent capillary sequencing, the tube containing the eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see below).

\* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

† If the samples are not diluted, use water to calibrate the spectrophotometer.

To quantify DNA isolated using the EZ1 system:

- Apply the tube containing the DNA to a suitable magnetic separator (e.g., QIAGEN 12-Tube Magnet, cat. no. 36912) for 1 min. If a suitable magnetic separator is not available, centrifuge the tube containing the DNA for 1 min at full speed in a microcentrifuge to pellet any remaining magnetic particles.
- Once separation is complete, carefully withdraw 10–50  $\mu\text{l}$  of isolated DNA and dilute to a final volume of 100  $\mu\text{l}$  in buffer of neutral pH.
- Measure the absorbance at 320 nm and 260 nm. Subtract the absorbance reading obtained at 320 nm from the reading obtained at 260 nm to correct for the presence of magnetic particles.

Concentration of DNA sample =  $50 \mu\text{g/ml} \times (A_{260} - A_{320}) \times \text{dilution factor}$

Total amount of DNA isolated = concentration  $\times$  volume of sample in ml

## Purity of DNA

Purity is determined by calculating the ratio of corrected absorbance at 260 nm to corrected absorbance at 280 nm, i.e.,  $(A_{260} - A_{320}) / (A_{280} - A_{320})$ . Pure DNA has an  $A_{260}/A_{280}$  ratio of 1.7–1.9. Use buffer of slightly alkaline pH (e.g., 10 mM Tris-Cl, pH 7.5) to dilute the samples and to calibrate the spectrophotometer. \*

\* If the samples are not diluted, use water to calibrate the spectrophotometer.

# Ordering Information

Product	Contents	Cat. no.
EZ1&2 DNA Blood 200 µl Kit (48)	48 reagent cartridges (Blood 200 µl), 50 disposable tip holders, 50 disposable filter-tips, 50 sample tubes (2 ml), 50 elution tubes (1.5 ml)	951034
EZ1&2 DNA Blood 350 µl Kit (48)	48 reagent cartridges (Blood 350 µl), 50 disposable tip holders, 50 disposable filter-tips, 50 sample tubes (2 ml), 50 elution tubes (1.5 ml)	951054
EZ2 Connect	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 1-year warranty on parts and labor.	9003210
<b>Related products</b>		
Filter-Tips and Holders (50)	50 Disposable Filter-Tips, 50 Disposable Tip Holders; additional tips and holders for use with EZ1&2 Kits	994900
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at [www.qiagen.com](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

# Document Revision History

Revision	Description
09/2022	Initial release

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### Limited License Agreement for EZ1&2 DNA Blood Kits

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at [www.qiagen.com](http://www.qiagen.com). Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

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