April 2025

EZ1&2™ DNA Investigator® Kit Handbook

For automated purification of DNA from forensic and human ID samples using the EZ2® Connect Fx instrument

Sample to Insight

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Kit Contents

EZ1&2 DNA Investigator Kit **Catalog no.** **No. of preps**	**(48)** **952034** **48**
Reagent Cartridge, DNA Investigator*	48
Disposable Tip Holders	50
Disposable Filter-Tips	50
Sample Tubes (2 mL)	50
Elution Tubes (1.5 mL)	50
Buffer G2	2 x 13 mL
Proteinase K	1 x 1.2 mL
Carrier RNA	1 x 310 µg
Q-Card†	1
Quick-Start Protocol	2

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See Safety Information section on page 6.

† The information encoded in the bar code on the Q-Card is needed for reagent data tracking on the EZ2 Connect Fx.

Shipping and Storage

The EZ1&2 DNA Investigator Kit is 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. Lyophilized carrier RNA is stable until the expiration date on the Q-Card when stored at room temperature.

Intended Use

The EZ1&2 DNA Investigator Kit is intended for molecular biology applications in forensics, human identity, and paternity testing. The EZ1&2 DNA Investigator Kit is intended to be used with EZ2 Connect Fx, EZ1® Advanced XL, EZ1 Advanced, and BioRobot® EZ1 instruments. This product is neither intended for the diagnosis, prevention, or treatment of a disease, nor has it been validated for such use either alone or in combination with other products. 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** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

CAUTION DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffers in the reagent cartridges contain guanidine hydrochloride/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 1% (v/v) sodium hypochlorite, followed by water.

Quality Control

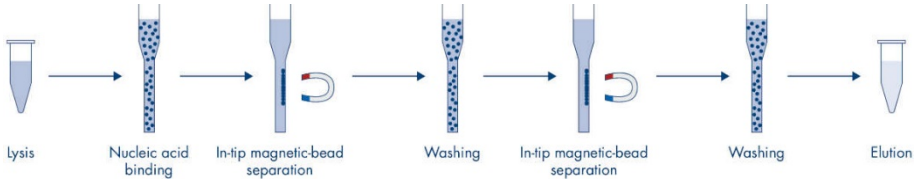
In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EZ1&2 DNA Investigator Kit is tested against predetermined specifications to ensure consistent product quality. Functional QC testing ensures that the EZ1&2 DNA Investigator Kit meets the high standards required by forensic scientists. The EZ1&2 DNA Investigator Kit meets ISO 18385 requirements.

Introduction

The EZ2 Connect Fx instrument and the EZ1&2 DNA Investigator Kit reproducibly automate purification of genomic DNA from samples encountered in forensic, human identity, and biosecurity applications. Magnetic-particle technology provides high-quality DNA that is suitable for direct use in downstream applications, such as quantitative real time PCR, STR analysis, or NGS applications. The EZ2 Connect Fx instrument performs all steps of the sample preparation procedure, and the user can choose sample input volumes of 200 µL, 500 µL, or 2 mL, 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 (see the flowchart below). DNA is isolated from lysates in one step through its 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 in the user's choice of either water or TE buffer. The user can choose elution volumes between 20 µL and 200 µL.



Description of protocols

This handbook contains 2 types of protocols (Table 1 on page 12).

- Pretreatment protocols detail the preliminary steps, such as Proteinase K digestion, prior to processing on the EZ2 Connect Fx instrument.
- DNA purification protocols describe setting up the EZ2 Connect Fx instrument and starting a fully automated run.

Pretreatment protocols

Since the type of samples that can be processed using the EZ1&2 DNA Investigator Kit can vary greatly, there is also a variety of different pretreatments, optimized for specific sample types.

DNA purification protocols

There are 11 DNA purification protocols, which can be used in conjunction with the pretreatment protocols. Within each protocol, the user can specify elution in water or TE buffer, with elution volumes of 20–200 µL.

DNA Purification: Trace Protocol on page 40. This protocol can be used with all various types of samples that can efficiently be lysed in a volume of 200 µL.

DNA Purification: "Tip Dance" Protocol on page 43. In this protocol, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, fabrics, blood discs, or cigarette butts, directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or if the sample material is precious. The protocol can be used for samples that are expected to give sufficient yield. To maximize yields of critical samples, we recommend the Investigator Lyse&Spin Basket Kit and the Large-Volume Protocols to recover DNA from the entire lysate.

The Large-Volume Protocols enable fully automated processing of starting volumes up to 500 µL. This allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, as well as purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes, with the same elution volume as the standard Trace Protocol, enables higher yields of more concentrated DNA for greater sensitivity in downstream applications. The Large-Volume Protocols are used for samples processed with the Investigator Lyse&Spin Basket Kit.

The EZ2 Connect Fx instrument allows the user to choose between 3 versions of the Large-Volume Protocol.

DNA Purification: DNA Large-Volume Protocol on page 46. This protocol is equivalent to the Large-Volume Protocol on EZ1 instruments. It requires the manual addition of 400 µL Buffer MTL to the sample lysate. It does not make use of the buffer filled in well 10 of the reagent cartridge. The protocol can be used for equivalence testing purposes between EZ1 and EZ2 instruments.

DNA Purification: DNA Large-Volume Protocol RT on page 49. This is a modified version of the DNA Investigator Large-Volume Protocol. For the DNA Investigator Large-Volume Protocol RT, the user does not need to manually add additional MTL buffer to the sample tube. The EZ2 Connect Fx instrument will add room temperature (15–25°C) MTL buffer, from well 10 of the reagent cartridge, directly to the sample tube.

DNA Purification: DNA Large-Volume Protocol Heated on page 52. This is a modified version of the DNA Investigator Large-Volume Protocol. For the DNA Investigator Large-Volume Protocol Heated, the user does not need to manually add additional MTL buffer to the sample tube. The EZ2 Connect Fx instrument will heat MTL buffer, from wells 1 and 10 of the reagent cartridge, and add this directly to the sample tube.

DNA Purification: Normalization Protocol on page 56. This protocol is designed for isolation of defined quantity of DNA by limiting the maximum binding capacity. The protocol can be used to obtain uniform yields from samples containing excess of DNA, e.g., buccal swabs taken as a reference sample.

DNA Purification: Normalization "Tip Dance" Protocol on page 59. This protocol combines the Normalization Protocol and the "Tip Dance" Protocol to allow processing of solid sample substrates.

DNA Purification: 1.5 mL Sample Tube on page 62. This protocol uses the QIAcube 1.5 mL elution tube as sample input. It allows to do the extraction from a sperm fraction obtained with the automated differential wash protocol without transferring the lysate to a 2 mL sample tube.

DNA Purification: RNA/DNA Co-Extraction on page 65. This protocol is a modification of the Large Volume Protocol. It binds in the presence of alcohol, which allows for co-extraction of RNA. DNA yields stay the same, DNA and RNA are eluted into the same tube. 400 µL of isopropanol, and 360 µL of ethanol have to be added to the sample before starting the run.

DNA Purification: Bone Extra-Large Volume Protocol on page 68. This protocol purifies DNA from 2 mL lysate. It can be used to extract larger amounts of bone powder, or from bulky samples that may require a larger lysate volume, such as cartridge casings. The protocol requires the use of the Large Volume Rack on the instrument.

DNA Purification: Firearms and Cartridge Casing Protocol on page 71. This protocol is designed for the isolation of DNA from firearms, fired and unfired cartridge casings that have been pretreated as described in the relevant protocol in this handbook on page 34.

Table 1. Protocol information for different sample types

Sample type	Pretreatment protocols	Purification protocols	Sample amount	Buffer G2	Proteinase K	DTT 1 M
Blood/saliva	page 23	Trace	Up to 50 µL	140–190 µL	10 µL	no
FTA® cards	page 23	Trace or Tip Dance	4 x 3 mm punches	290 µL	10 µL	no
Surface swabs	page 23	Trace or Tip Dance	1 swab	290 µL	10 µL	no
Chewing gum	page 23	Trace or Tip Dance	Up to 40 mg	190 µL	10 µL	no
Cigarette butts	page 23	Trace or Tip Dance	1 cm^2	190 µL	10 µL	no
Paper/similar materials	page 23	Trace or Tip Dance	0.5–2.5 cm^2	190 µL	10 µL	no
Nail scrapings	page 23	Trace	Up to 40 mg	190 µL	10 µL	no
Nail clippings	page 23	Trace	1	160 µL	20 µL	20 µL
Hair	page 23	Trace	0.5–1 cm	160 µL	20 µL	20 µL
Tissues	page 23	Trace	Up to 10 mg	190 µL	10 µL	no
Blood or saliva stains	page 23	Trace or Tip Dance	0.5 cm^2	290 µL	10 µL	no
Semen stains	page 23	Trace or Tip Dance	0.5 cm^2	270 µL	10 µL	20 µL
Large volume	page 23	Large-Volume	Varies	475 µL	25 µL	no
Large-volume semen	page 23	Large-Volume	Varies	455 µL	25 µL	20 µL
Sexual assault samples	page 26	Trace	Varies	Up to 2.5 mL*	20 µL	40 µL
Bones or teeth	page 29	Large-Volume	150–200 mg	225 µL	25 µL	no
Bones or teeth	page 31	Bone Extra-Large Volume	Up to 800 mg	900 µL	100 µL	no

Table 1. Protocol information for different sample types (continued)

Sample type	Pretreatment protocols	Purification protocols	Sample amount	Buffer G2	Proteinase K	DTT 1 M
Fired cartridge casing	page 34	Firearms and Cartridge Casing	Up to 2 mL	944 µL	56 µL	no
Firearm swabbing	page 37	Firearms and Cartridge Casing	Up to 2 mL	1888 µL	112 µL	no

* Depends on number of sperm pellet washes.

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

- Thermomixer, heating block, or water bath
- Vortexer
- Pipettes and pipette tips (to prevent cross-contamination, we strongly recommend the use of pipette tips with aerosol barriers)

For purification of DNA from epithelial cells mixed with sperm cells

- Buffer G2 (cat. no. 1014636)
- 1 M dithiothreitol (DTT), forensic grade quality (cat. no. 1117316)
- Microcentrifuge

For purification of DNA from hair

- 1 M dithiothreitol (DTT), forensic grade quality

For purification of DNA from bones or teeth using the Large-Volume Protocol

- 0.5 M EDTA, pH 8.3
- Liquid nitrogen
- 3 M sodium acetate (NaOAc), pH 5.0
- Centrifuge
- Thermal mixer or orbital incubator
- TissueLyser III (cat. no. 9003240), with the Grinding Jar Set, S. Steel (cat. no. 69985), or an equivalent bead mill

For purification of DNA from bones or teeth using the Bone Extra-Large Volume Protocol

- 0.5 M EDTA, pH 8.3
- Liquid nitrogen
- 3 M sodium acetate (NaOAc), pH 5.0
- Centrifuge
- Thermal mixer or orbital incubator
- TissueLyser III (cat. no. 9003240), with the Grinding Jar Set, S. Steel (cat. no. 69985), or an equivalent bead mill
- EZ2 Connect Tip Rack, Large Volume (cat. no. 9027011)
- Large-Volume Tubes 7 mL (cat. no. 951954)

- Buffer MTL (54 mL) (cat. no. 19112)
- Buffer G2 (260 mL) (cat. no. 1014636)
- QIAGEN Proteinase K (2 mL) (cat. no. 19131)
- 100% Ethanol
- Suitable tube for lysis (e.g., 15 mL Falcon Tube, 7 mL Large-Volume Tube)
- Centrifuge for lysis tube

For DNA purification, Large-Volume Protocol

- Buffer MTL (54 mL) (cat. no.19112)

For DNA purification, Large-Volume Protocol, Heated

- 2 mL Sarstedt tubes (cat. no. 72.693.005)

For DNA purification, RNA/DNA Co-Extraction

- 100% Ethanol
- Isopropanol

For pretreatment of samples from firearms and cartridge casing

- Buffer G2 (260 mL) (cat. no. 1014636)
- 100% ethanol, absolute (200 proof), molecular biology grade, Fisher BioReagents™ (cat. no. BP2818-4) (or alternative)
- Graduated, flat-base 5 mL tubes (cat. no. 990552)
- Large-Volume Tubes 7 mL (cat. no. 951954)
- Kelly Forceps (straight, serrated tip or similar)
- 4N6FLOQSwabs in peelpouch (cat. no. WB100100)
- Vortexer
- Thermomixer or alternative incubator
- EZ2 Connect Tip Rack - Large Volume (cat. no. 9027011)

Important Notes

Starting material

The amount of starting material for use in EZ1&2 DNA Investigator procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts is given in their respective protocols and in Table 1 on page 12. The EZ2 Connect Fx instrument can process 200 µL of pretreated sample using the Trace Protocol on page 40 or the "Tip Dance" Protocol on page 43 for DNA purification. With the Large-Volume Protocol on page 46, up to 500 µL of pretreated sample can be processed. The Bone Extra-Large Volume Protocol on page 68 allows to extract from 2 mL sample lysate.

Purification of low amounts of DNA

For purification of DNA from very small amounts of sample, such as low volumes of blood (<10 µL) or forensic casework samples, we recommend adding carrier RNA. For samples containing larger amounts of DNA, addition of carrier RNA is optional. Add 310 µL TE buffer or water to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µL. Dissolve the carrier RNA thoroughly, divide into conveniently sized aliquots, and store at –30°C to –15°C. Do not freeze-thaw the aliquot of carrier RNA more than 3 times. Carrier RNA should be added to the sample after the lysis is completed to avoid degradation.

Precipitate in Reagent Cartridge

The buffer in well 1 (the well nearest to the front of the EZ2 Connect Fx instrument when the reagent cartridge is loaded) and well 10 of the reagent cartridge may form a precipitate upon storage. If necessary, redissolve by mild agitation at 37°C and then place at room temperature (15–25°C).

Equilibrating Reagent Cartridges

If reagent cartridges have been stored at 2–8°C, they must be equilibrated to operating temperature before use. Place the reagent cartridge into a shaker–incubator and incubate at 30–40°C with mild agitation for at least 2 hours before use. If precipitates are visible at the bottom of the wells, redissolve by incubating at 30–40°C with mild agitation for an additional 2 hours. Do not use the reagent cartridges if the precipitates do not redissolve.

Lysis with Proteinase K

The EZ1&2 DNA Investigator Kit contains Proteinase K, which is the enzyme of choice for lysis buffers used in DNA Investigator protocols. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and is particularly suitable for short digestion times. It possesses a high specific activity and remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. The activity of the Proteinase K solution is 600 mAU/mL solution (or 40 mAU/mg protein). This activity provides optimal results in DNA Investigator protocols.

Quantification of DNA

Degraded, inhibited, or mixed DNA samples are common in forensic casework and other human identity testing applications. Such samples can create challenges in STR analysis. Prior quantification of the purified DNA using real-time PCR is recommended and reduces the need to repeat downstream analyses. This greatly reduces costs and time and improves the statistical relevance of results. Investigator Quantiplex® Pro Kits use quantitative real-time PCR to quantify human total and human male DNA in a sample. These kits also detect if the sample contains inhibitors that may interfere with downstream applications, or if the DNA is degraded.

EZ2 Connect Fx Reagent Cartridges

Reagents for the purification of nucleic acids from a single sample are contained in a single reagent cartridge (Figure 1). Each well of the cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Details on preparation of these positions are displayed during the run setup on the LED display of the EZ2 Connect Fx.

Figure 1. 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.

EZ2 Connect Fx Tip Racks

The EZ2 Connect Fx comes with 2 different types of Tip Racks. One is compatible with screw cap tubes, and one is compatible with flip-cap tubes. The EZ2 Connect Fx tip racks hold tips inserted into tip holders and tubes for samples or elution (Figure 2). Details on how to equip the tip racks are displayed during the run setup on the LED display of the EZ2 Connect Fx.

Figure 2. EZ2 Connect Fx tip racks, tip holders, and filter-tips.

Worktable

The worktable of EZ2 Connect Fx instruments is where the user loads cartridges and tip racks (Figure 3 on the facing page).

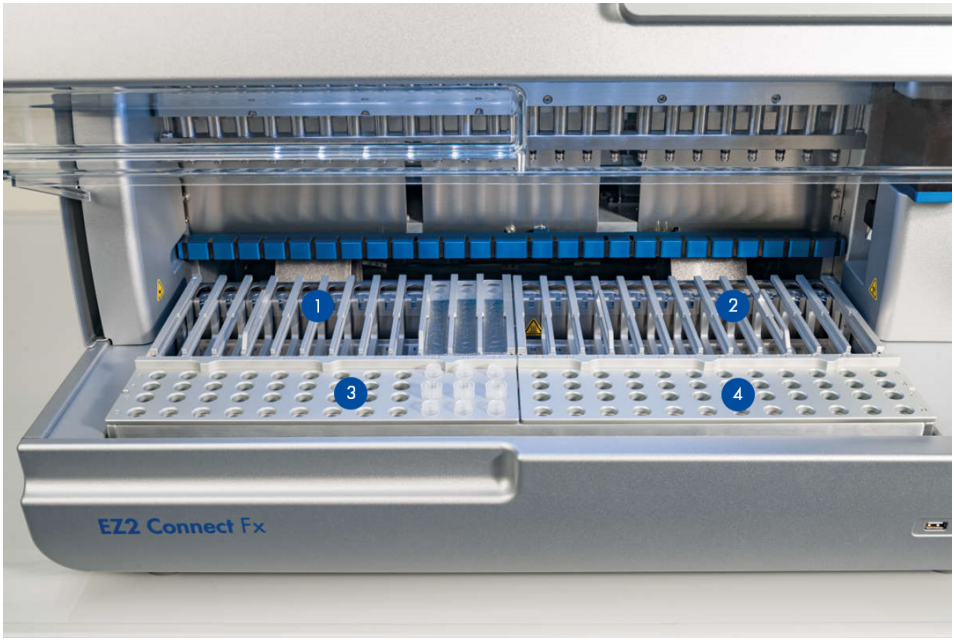


Figure 3. EZ2 Connect Fx Worktable. (1) EZ2 Connect Cartridge Rack – left. **(2)** EZ2 Connect Cartridge Rack – right. **(3)** EZ2 Connect Tip Rack – left. **(4)** EZ2 Connect Tip Rack – right.

Operation of the EZ2 Connect Fx

The EZ2 Connect Fx provides various features to support the sample preparation workflow. These include functions for remote access via QIAsphere®, data input via bar code reading, load check, data storage and transfer, report generation, and guided instrument maintenance.

The EZ2 Connect Fx has preinstalled protocols that are used with the EZ1&2 DNA Investigator Kit to purify nucleic acids. The instrument's touchscreen display allows the user to easily select protocols. The intuitive user interface of the software guides the user through the run setup

process, which includes selecting variable parameters. The display also shows protocol status during the automated purification procedure.

The aspiration and dispensation of samples and reagents and the separation of magnetic particles are performed by the 24-channel pipettor head. If required by the protocol, the temperature of the liquids is controlled by the heating system.

For more information about these features, refer to the *EZ2 Connect and EZ2 Connect Fx User Manual*.

Pretreatment Protocol for Various Casework and Reference Samples

This protocol is designed for isolation of total DNA (genomic and mitochondrial) from various types of casework and reference samples. The protocol describes the preliminary lysis using Proteinase K.

Important points before starting

- Before beginning the procedure, read “Important Notes” on page 17.
- We recommend using the Investigator Lyse&Spin Basket Kit (cat. no. 19597 or 19598) when solid sample materials have to be removed from the lysate. If using this kit, follow the Pretreatment Protocol described in the corresponding handbook and the Large-Volume Protocols for DNA purification. The collection tubes from the Investigator Lyse&Spin Kit can be used as sample tubes for the EZ2 Connect Fx, using the Flip-Cap Tube Rack.

Thing to do before starting

- Heat a thermomixer, heating block, or water bath to 56°C for the Proteinase K digest in step 3.

Procedure

1. Place the sample in a 2 mL sample tube.
2. Set up the Proteinase K digest according to information given in Table 1 on page 12. Mix sample thoroughly by vortexing for 10 s.
3. Incubate at 56°C for 15 min to overnight in a thermomixer shaking at 900 rpm.

   Fifteen minutes may be sufficient to recover adequate DNA for STR typing from samples containing abundant DNA. More than 1 h is recommended where a low amount of DNA is expected.
4. If necessary, flick the tube to remove drops from inside the lid.

   Optional: Add 1 µg carrier RNA (see "Important Notes" on page 17)
5. Continue with DNA Purification using one of the following options:

   a. **DNA Purification: Trace Protocol** on page 40.

      For samples that do not contain solid materials. The lysate volume should be approximately 200 µL.

   b. **DNA Purification: "Tip Dance" Protocol** on page 43.

      When using the "Tip Dance" Protocol, there is generally no need to remove solid material from the tube. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or if the sample material is precious. Note that the "Tip Dance" Protocol will not recover the lysate absorbed by the sample substrate (e.g., swab, piece of fabric); therefore, a slightly reduced sensitivity has to be expected compared to methods that fully recover the lysate. We recommend using the Investigator Lyse&Spin Basket Kit for maximum sensitivity.

c. **DNA Purification: DNA Investigator Large-Volume Protocol** on page 46.

The Large-Volume Protocols purify DNA from 500 µL lysate.

Pretreatment Protocol for Epithelial Cells Mixed with Sperm Cells

This protocol is designed for purification of total (genomic and mitochondrial) DNA from epithelial cells mixed with sperm cells. The protocol describes the preliminary lysis of samples using Proteinase K and dithiothreitol (DTT).

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 17.
- This protocol requires materials not included in the EZ1&2 DNA Investigator Kit, which need to be purchased separately. Please refer to "Equipment and Reagents to Be Supplied by User" on page 13.
- As some sample types (e.g., fabrics) tend to be very absorbent, it may be necessary to add a greater volume of digestion buffer to the sample in step 2.

Thing to do before starting

- Heat a thermomixer, heating block, or water bath for the Proteinase K digest to 56°C in step 4 and 70°C in step 14. Incubate at 70°C for 10 min at 850 rpm in a shaker–incubator or thermomixer.

Procedure

1. Place the forensic sample in a 1.5 mL or 2 mL sample tube.
2. Add 190 µL Buffer G2 to the sample.
3. Add 10 µL Proteinase K, and mix thoroughly by vortexing for 10 s.
4. Incubate at 56°C for 1–2 h. Do not exceed 2 h.
5. Vortex the tube once or twice during the incubation, or place in a thermomixer.
6. Centrifuge the tube briefly to remove drops from inside the lid.
7. Remove any solid material from the tube.
8. Centrifuge the tube at 15,000 x *g* for 5 min. Carefully transfer the supernatant to a new tube without disturbing the sperm cell pellet.
9. DNA from epithelial cells can be purified from the tube containing the supernatant following "DNA Purification: Trace Protocol" on page 40, or if the epithelial cell fraction is very dilute, "DNA Purification: Large-Volume Protocol" on page 46.

   Note: The cell pellet may not be visible.
10. Wash the sperm cell pellet by resuspending the pellet in 500 µL Buffer G2. Centrifuge the tube at 15,000 x *g* for 5 min and discard the supernatant.
11. Repeat step 10 two or three times.
12. Add 160 µL Buffer G2 to the pellet and resuspend the pellet.
13. Add 10 µL Proteinase K and 40 µL 1 M DTT, and mix thoroughly by vortexing for 10 s.
14. Incubate at 70°C for 10 min at 850 rpm in a shaker–incubator or thermomixer.
15. For maximum recovery, place samples in an Ultrasonicator for 10 min. Alternatively, vortex vigorously for 10 s.

16. Centrifuge the tube briefly to remove drops from inside the lid. DNA from sperm cells can now be purified from this tube.
17. Continue with "Protocol: DNA Purification (Trace Protocol)" on page 40. The 2 tubes in which the epithelial and sperm cells have been separated are now ready for DNA purification.

Pretreatment Protocol of Bone or Teeth

This protocol is designed to enable efficient recovery of inhibitor-free DNA from bone and teeth samples using the Large-Volume Protocol. The protocol describes the disruption and lysis of samples using Proteinase K and EDTA.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read "Important Notes" on page 17.
- This protocol requires materials not included in the EZ1&2 DNA Investigator Kit, which need to be purchased separately. Please refer to "Equipment and Reagents to Be Supplied by User" on page 13.

Thing to do before starting

- Heat a thermal mixer or orbital incubator to 56°C for the Proteinase K digest in step 7.

Procedure

1. Remove and discard the bone or teeth surfaces. Grind the remaining bone or tooth root to a fine powder.

Note: Grind using a metal blender half-filled with liquid nitrogen. Alternatively, grind using the TissueLyser III and the Grinding Jar Set, S. Steel.

Note: When using the TissueLyser III, transfer the bone or tooth sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone or tooth fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser III. Grind the bone at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of sample).

2. Place up to 150 mg of powdered bone into a 2 mL sample tube. Do not exceed the amount of bone powder. If higher overall yields are required, we recommend processing additional extractions and to pool and concentrate the eluates.
3. Add 225 µL Buffer G2.
4. Add 25 µL Proteinase K.
5. Add 250 µL 0.5 M EDTA, pH 8.0.
6. Mix by inverting the 2 mL tube several times.
7. Place the tube into the thermal mixer or heated orbital incubator, and incubate with constant motion at 56°C for at least 2 h and up to 24 h.
8. Centrifuge at 6000 rpm for 4 min to pellet any remaining debris. Transfer the supernatant to a new 2 mL sample tube.
9. Add 50 µL 3 M NaOAc, pH 5.0, to each 2 mL sample tube.
10. Add 1 µL carrier RNA to each 2 mL sample tube.
11. Continue with “DNA Purification: Large-Volume Protocol” on page 46; “DNA Purification: Large-Volume Protocol RT” on page 49; or “DNA Purification: Large-Volume Protocol Heated” on page 52.

Pretreatment Protocol of Bone or Teeth for Extraction with the Bone Extra-Large Volume Protocol

This protocol is designed to enable efficient recovery of inhibitor-free DNA from bone and teeth samples using the Bone Extra-Large Volume Protocol. The protocol describes the disruption and lysis of samples using Proteinase K and EDTA.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read "Important Notes" on page 17.
- This protocol requires materials not included in the EZ1&2 DNA Investigator Kit, which need to be purchased separately. Please refer to "Equipment and Reagents to Be Supplied by User" on page 13.
- The Large Volume EZ2 Connect Tip Rack is not included with the purchase of an EZ2 Connect Fx and must be purchased separately.
- Up to 800 mg of bone powder can be used as sample. For most fresh and old bone samples tested, yields did not further increase with more than 400 mg. Do not exceed the amount of bone powder. If higher overall yields are required, we recommend processing additional extractions and to pool and concentrate eluates.
- Two to four hours of lysis has been found to provide optimal results. Incubations exceeding 6 hours can lead to significantly reduced yields. We do not recommend overnight lysis.

Thing to do before starting

- Heat a thermal mixer or orbital incubator to 56°C for the Proteinase K digest in step 7.

Procedure

1. Remove and discard the bone or teeth surfaces. Grind the remaining bone or tooth root to a fine powder.

   Note: Grind using a metal blender half-filled with liquid nitrogen. Alternatively, grind using the TissueLyser III and the Grinding Jar Set, S. Steel.

   Note: When using the TissueLyser III, transfer the bone or tooth sample and the ball into the grinding jar. Pour liquid nitrogen into the grinding jar over the ball and bone or tooth fragments. Allow the temperature to equilibrate (i.e., liquid nitrogen stops boiling). Decant the excess liquid nitrogen, close the grinding jar with the lid, and transfer it to the TissueLyser III. Grind the bone at 30 Hz for 1 min or until the bone is pulverized (grinding times depend on type, condition, and size of sample).

2. Place 100–800 mg of powdered bone into a suitable sample tube.

   Note: the sample tube should be compatible with your lab's thermomixer and centrifuge in step 7 and be able to accommodate volumes in the protocol.

3. Add 900 µL Buffer G2.
4. Add 100 µL Proteinase K.
5. Add 1000 µL 0.5 M EDTA, pH 8.0.
6. Mix by inverting the tube several times.

7. Place the tube in a thermomixer or heated orbital incubator, and incubate with constant motion at 56°C for 2–6 hours. Centrifuge the tube for 5 min at approximately 1900 x *g* to pellet remaining debris. The centrifugal speed required is dependent on the centrifuge and rotor.

   Note: If the volume of lysate recovered is lower than 2 mL, add buffer a 1:1 mixture of Buffer G2 and EDTA to a final volume of approximately 2 mL.

8. Transfer the supernatant to a new 7 mL sample tube.
9. Add 1000 µL Buffer MTL and 700 µL ethanol to the 7 mL sample tube. Buffer MTL must be added to the digested samples when still warm to prevent precipitation.
10. Add 160 µL 3 M NaOAC, pH 5.0, to the 7 mL sample tube.
11. Continue with "DNA Purification: Bone Extra-Large Volume Protocol" on page 68.

Pretreatment Protocol for Firearms and Cartridge Casing (FCC)

This protocol is intended for the lysis and extraction of DNA from forensic samples of fired (casings) and unfired (cartridges) ammunition. The "DNA Purification: Firearms and Cartridge Casing" protocol may be used such that swabbings and lysates of 2 fired or unfired cartridge casings may be combined into one single extraction tube.

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 17.
- If using the EZ1&2 DNA Investigator Kit for the first time, please read the "Safety Information" on page 6.
- The Large Volume EZ2 Connect Tip Rack is not included with the purchase of an EZ2 Connect Fx and must be purchased separately. Please refer to "Equipment and Reagents to Be Supplied by User" on page 16.

Thing to do before starting

- Pre-heat a thermomixer or incubator to 56°C for the Proteinase K digestion in step 4.

Procedure: Fired cartridge casing

The lysate from 2 fired cartridge casings (FCC), totaling 2 mL, may be combined for extraction on the EZ2 Connect Fx using the DNA purification protocol for firearms and cartridge casing.

1. Place each individual fired cartridge casing upright into a graduated, flat base 5 mL tube such that the closed end of the casing is seated in the bottom of the tube.
2. Prepare 1 mL lysis buffer volume per casing.

Reagent	Volume per sample (µL)
Buffer G2	944
Proteinase K	56

3. Pipette 1000 µL of Buffer G2/Proteinase K lysis buffer to each individual casing. Casing will be almost submerged.

   Important: Pipette lysis buffer specifically between the outer surface of the casing and the inside wall of the 5 mL tube. Do not pipette inside the casing.

   Note: This pre-treatment protocol was developed using fired 9 mm casings. If a different caliber is used, be observant that the buffer does not enter the open casing.
4. Avoid buffer from entering the casing and incubate at 56°C for 30 minutes.
5. Hold the Kelly forceps in a vertical position and remove the casing from the lysis buffer.

   Note: Avoid tilting the cartridge casing in such a way that any lysis buffer sitting on the inside may pour out.
6. Thoroughly swab the casing's outside surface, discard the casing, and return the head of the swab to the same 5 mL tube containing the respective lysis buffer.
7. Obtain a second sample tube and repeat step 5.
8. Thoroughly swab the outside surface of the second casing, discard the casing, and return the head of the second swab to the first sample tube.
9. Transfer the lysate from the second tube to the first tube.

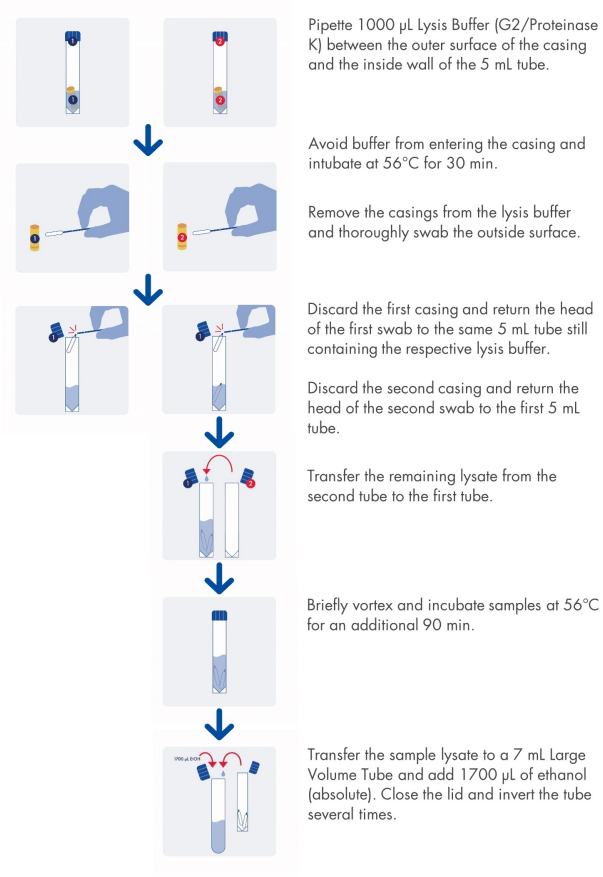


Figure 4. Pretreatment workflow for fired cartridge casing.

10. The total volume should be approximately 2 mL of lysate. If only one casing is being processed (1 mL lysate), add Buffer G2 to a total volume of 2 mL.
11. Briefly vortex and incubate samples at 56°C for an additional 90 min.
12. Transfer the sample lysate to a new 7 mL sample tube and pipette 1700 µL of ethanol into each 7 mL sample tube.
13. Continue with “DNA Purification: Firearms and Cartridge Casing Protocol” on page 71.

Procedure: Firearm swabbing

1. Prepare the lysis buffer by mixing 1888 µL Buffer G2 and 112 µL Proteinase K into a graduated, flat base 5 mL tube.
2. Thoroughly swab the surface of the firearm with 1 or 2 dry swabs and 1 or 2 moistened swabs at relevant locations such as the handle, slide, trigger, magazine slice clip.

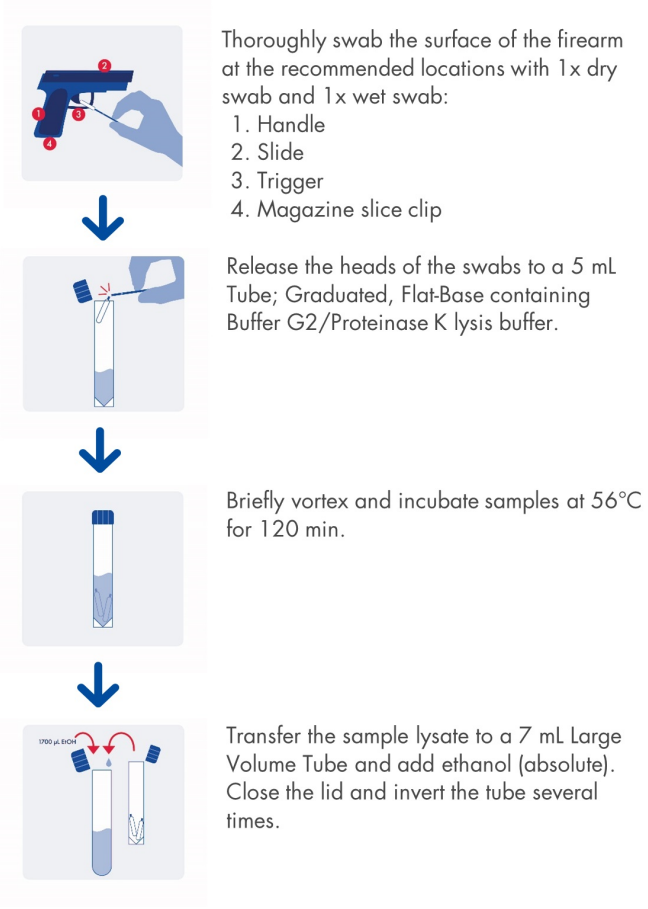


Figure 5. Pretreatment protocol for firearm swabbings.

3. Release the heads of the swabs into the 5 mL tube containing 2000 µL lysis buffer.
4. Briefly vortex and incubate samples at 56°C for 120 min.

5. Transfer the sample lysate to a large-volume tube 7 mL and add 1700 µL of ethanol. Pipette up and down to mix.
6. Continue with “DNA Purification: Firearms and Cartridge Casing Protocol” on page 71.

DNA Purification: Trace Protocol

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" on page 23 to 28). The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- The reagent cartridges contain guanidine salts and are therefore not compatible with disinfecting reagents containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See “Equilibrating Reagent Cartridges” on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If

necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel, select "DNA Investigator Kit", and then select **Next**.
3. Choose the protocol "DNA Investigator Trace" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
9. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack):

   Standard Tip Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Empty
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube

Flip-Cap Rack

- Position A: Opened 2.0 mL tube with sample
- Position B: Tip holder with inserted tip
- Position C: Opened 1.5 mL empty elution tube

Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.
11. The display will show "Protocol finished" when the run is completed. Select **Finish**.
12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: “Tip Dance” Protocol

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" on page 23 to 28). In the “Tip Dance” Protocol, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials such as swabs, fabrics, blood discs, or cigarette butts directly in the sample tube. There is generally no need for prior centrifugation to remove solid materials that could clog the tip. However, when processing fluffy sample material such as cotton wool, we recommend removing solid material if you cannot process a replicate sample or the sample material is precious. The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Tip Dance" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
9. Prepare the EZ2 Connect Tip Rack as follows (Position labels are engraved on the EZ2 Connect Tip Rack):

Standard Tip Rack

- Position A: Opened 2.0 mL tube with sample
- Position B: Empty
- Position C: Tip holder with inserted tip
- Position D: Opened 1.5 mL empty elution tube

Flip-Cap Rack

- Position A: Opened 2.0 mL tube with sample
- Position B: Tip holder with inserted tip
- Position C: Opened 1.5 mL empty elution tube

Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.
11. The display will show "Protocol finished" when the run is completed. Select **Finish**.
12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: Large-Volume Protocol

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" on page 23 to 28). The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Starting material

Using this protocol, up to 500 µL of pretreated sample can be processed. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes, with the same elution volume as the standard Trace Protocol, enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).
- Add 400 µL Buffer MTL to each sample lysate. The protocol does not make use of buffer MTL contained in well 10 of the reagent cartridge. For automated addition, choose the "Large-Volume RT" or the "Large-Volume Heated" protocol.

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Large Volume" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.

8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
9. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack):

   Standard Tip Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Empty
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube

   Flip-Cap Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Tip holder with inserted tip
   - Position C: Opened 1.5 mL empty elution tube

   Select **Next**.
10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.
11. The display will show "Protocol finished" when the run is completed. Select **Finish**.
12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube. Note that liquid has been wasted into the sample tube during the extraction process.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: Large-Volume Protocol RT

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" on page 23 to 28). The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Starting material

Using this protocol, up to 500 µL of pretreated sample can be processed. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes, with the same elution volume as the standard Trace Protocol, enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Large Volume RT" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.

9. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack):

   Standard Tip Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Empty
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube

   Flip-Cap Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Tip holder with inserted tip
   - Position C: Opened 1.5 mL empty elution tube

   Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.

11. The display will show "Protocol finished" when the run is completed. Select **Finish**.

12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube. Note that liquid has been wasted into the sample tube during the extraction process.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.

13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: Large-Volume Protocol Heated

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" on page 23 to 28). The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Starting material

Using this protocol, up to 500 µL of pretreated sample can be processed. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes, with the same elution volume as the standard Trace Protocol, enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- The protocol requires an additional tube to be loaded to the heating block. Only 2 mL Sarstedt tubes (cat. no. 72.693.005) without a rim can be used in this position. Note that the sample tubes provided with the EZ1&2 DNA Investigator Kit cannot be used in the

heating block.

- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Large Volume Heated" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.

5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Load opened 2 mL Sarstedt tubes (cat. no. 72.693.005) into the heating block position of the cartridge.
8. Select **Next**.
9. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
10. Prepare the EZ2 Connect Tip Rack as follows (Position labels are engraved on the EZ2 Connect Tip Rack):

    Standard Tip Rack

    - Position A: Opened 2.0 mL tube with sample
    - Position B: Empty
    - Position C: Tip holder with inserted tip
    - Position D: Opened 1.5 mL empty elution tube

    Flip-Cap Rack

    - Position A: Opened 2.0 mL tube with sample
    - Position B: Tip holder with inserted tip
    - Position C: Opened 1.5 mL empty elution tube

    Select **Next**.
11. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load

check. If no load check is required, select **Skip load check** to start.

12. The display will show “Protocol finished” when the run is completed. Select **Finish**.
13. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube. Note that liquid has been wasted into the sample tube during the extraction process.

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

DNA Purification: Normalization Protocol

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" starting on page 23). The protocol results in the isolation of a defined quantity of DNA by limiting the maximum binding capacity. The protocol can be used to obtain uniform yields from samples containing excess of DNA, e.g., buccal swabs taken as a reference sample.

The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read "Important Notes" on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.

- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Normalization" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume. Select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
9. Prepare the EZ2 Connect Tip Rack as follows (Position labels are engraved on the EZ2 Connect Tip Rack):

   Standard Tip Rack

- Position A: Opened 2.0 mL tube with sample
- Position B: Empty
- Position C: Tip holder with inserted tip
- Position D: Opened 1.5 mL empty elution tube

Flip-Cap Rack

- Position A: Opened 2.0 mL tube with sample
- Position B: Tip holder with inserted tip
- Position C: Opened 1.5 mL empty elution tube

Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.
11. The display will show "Protocol finished" when the run is completed. Select **Finish**.
12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: Normalization “Tip Dance” Protocol

This protocol is designed for isolation of total (genomic and mitochondrial) DNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook (“Pretreatment Protocol for Various Casework and Reference Samples” starting on page 23). The protocol results in the isolation of a defined quantity of DNA by limiting the maximum binding capacity. The protocol can be used to obtain uniform yields from samples containing excess of DNA, e.g., buccal swabs taken as a reference sample. In the “Tip Dance” Protocol, the filter-tip moves back-and-forth relative to the worktable platform while pipetting. This enables processing of solid materials, such as swabs, directly in the sample tube.

The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Normalization Tip Dance" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.

9. Prepare the EZ2 Connect Tip Rack as follows (Position labels are engraved on the EZ2 Connect Tip Rack):

   Standard Tip Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Empty
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube

   Flip-Cap Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Tip holder with inserted tip
   - Position C: Opened 1.5 mL empty elution tube

   Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.

11. The display will show "Protocol finished" when the run is completed. Select **Finish**.

12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position 1 of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.

13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: 1.5 mL Sample Tube Protocol

This protocol is designed for isolation of DNA from sperm fractions obtained with the QIAcube differential wash protocols. The QIAcube 1.5 mL elution tube is used as sample tube. The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read "Important Notes" on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator 1.5 mL Sample Tube" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
9. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack). This protocol is only available for use with the

   Flip-Cap Rack

   - Position A: Opened 1.5 mL tube with sample
   - Position B: Tip holder with inserted tip
   - Position C: Opened 1.5 mL empty elution tube

   Select **Next**.
10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.

11. The display will show “Protocol finished” when the run is completed. Select **Finish**.
12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.
13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: RNA/DNA Co-Extraction

This protocol is designed for isolation of DNA and RNA from forensic samples that have been pretreated as described in the relevant protocols in this handbook ("Pretreatment Protocol for Various Casework and Reference Samples" on page 23 to 28). The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Starting material

Using this protocol, up to 500 µL of pretreated sample can be processed. This not only allows efficient DNA purification from dilute samples with low concentrations of DNA, such as diffuse stains, but also enables purification from samples that require larger volumes for thorough lysis. The ability to process larger sample volumes, with the same elution volume as the standard Trace Protocol, enables higher yields of more concentrated DNA for greater sensitivity in downstream applications.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read “Important Notes” on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator RNA/DNA Co-Extraction" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.

9. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack):

   Standard Tip Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Empty
   - Position C: Tip holder with inserted tip
   - Position D: Opened 1.5 mL empty elution tube

   Flip-Cap Rack

   - Position A: Opened 2.0 mL tube with sample
   - Position B: Tip holder with inserted tip
   - Position C: Opened 1.5 mL empty elution tube

   Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.

11. The display will show "Protocol finished" when the run is completed. Select **Finish**.

12. Open the instrument hood. Remove the elution tube containing purified nucleic acid from the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste, used tips and holders, and the sample tube. Note that liquid has been wasted into the sample tube during the extraction process.

    Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.

13. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: Bone Extra-Large Volume Protocol

This protocol is designed for isolation of DNA from larger amounts of powder from bones or teeth, or from bulky samples that may require a larger lysate volume, such as cartridge casings. The sample pretreatment is described in the relevant protocol in this handbook on page 31. The protocol describes the simple procedure for setting up the EZ2 Connect Fx instrument and starting a run.

Starting material

Using this protocol, up to 2 mL of lysate can be processed. This allows lysis and extraction from larger quantities, thereby increasing sensitivity for challenging samples.

Important points before starting

- If using the EZ1&2 DNA Investigator Kit for the first time, read "Important Notes" on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See safety information on page 6.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.
- The protocol requires the use of the Large-Volume Tip Rack.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Bone Extra Large Volume" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.

9. Prepare the EZ2 Connect Tip Rack as follows (position labels are engraved on the EZ2 Connect Tip Rack):

   Tip Rack – Large Volume

   - Position A: Tip holder with inserted tip
   - Position B: Large Volume Tube (7 mL), empty
   - Position C: Large Volume Tube (7 mL), contains sample
   - Position D: Opened 1.5 mL empty elution tube

   Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.

11. The display will show "Protocol finished" when the run is completed. Select **Finish**.

12. 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, used tips and holders, and the sample tube. Note that liquid has been wasted into the sample tube during the extraction process.

13. **Optional**: Follow onscreen instructions for UV decontamination of worktable surfaces.

    Perform regular maintenance after each run. Select **Finish** to return to the home screen.

DNA Purification: FCC Protocol

Starting material

Using this protocol, up to 2 mL of lysate can be processed. This allows lysis and extraction from larger quantities, thereby increasing sensitivity for challenging samples.

Important points before starting

- Before beginning the procedure, read "Important Notes" on page 17.
- Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 6 for Safety Information.
- Perform all steps of the protocol at room temperature (15–25°C). Work quickly during the setup procedure.
- The protocol requires the use of the Large-Volume Tip Rack.

Things to do before starting

- If reagent cartridges have been stored at 2–8°C, equilibrate to operating temperature before use. See "Equilibrating Reagent Cartridges" on page 18.
- Remove any solid material from the sample tube.
- The lysis buffer in the reagent cartridge may form a precipitate during storage. If

necessary, redissolve by warming at 37°C, and then place at room temperature (15–25°C).

Procedure

1. Turn on the EZ2 Connect Fx instrument.
2. Tap **DNA** on the Applications panel and then select "DNA Investigator Kit" and select **Next**.
3. Choose the protocol "DNA Investigator Firearm and Cartridge Casings" and select **Next**.
4. Choose the tip rack type, the elution buffer, and the elution volume and select **Next**.
5. Select positions on the work deck according to the number of samples to be processed and select **Next**.
6. Enter sample IDs or select **Generate missing sample IDs**. Then select **Next**.
7. Load the EZ1&2 DNA Investigator reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack as selected in step 5. Select **Next**.
8. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect Fx instrument.
9. Prepare the EZ2 Connect Tip Rack as follows (Position labels are engraved on the EZ2 Connect Tip Rack):

   Tip Rack – Large Volume

   - Position A: Tip holder with inserted tip
   - Position B: Large-Volume Tube (7 mL), empty

- Position C: Large-Volume Tube (7 mL), contains sample
- Position D: Opened 1.5 mL empty elution tube

Select **Next**.

10. Place the EZ2 Connect Tip Rack into the EZ2 Connect Fx instrument and start the run according to the instructions on the instrument display. The instrument will perform a load check. If no load check is required, select **Skip load check** to start.
11. The display will show "Protocol finished" when the run is completed. Select **Finish**.
12. 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, used tips and holders, and the sample tube. Note that liquid has been wasted into the sample tube during the extraction process.
13. **Optional**: Follow onscreen instructions for UV decontamination of worktable surfaces. Perform regular maintenance after each run. Select **Finish** to return to the home screen.

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 at our Technical Support Center: **www.qiagen.com/FAQ/FAQList.aspx** (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
General handling	
Error message in instrument display	Refer to the user manual supplied with your EZ2 Connect Fx instrument.
Reagent cartridge handling	
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, it is important that buffer volumes in the reagent cartridges are correct and that the filter tips fit optimally to the tip adapter. Ensure that samples are thoroughly mixed and that reagent cartridges have not passed their expiry date. Perform regular maintenance as described in the instrument user manual. Check the fit of the filter tips regularly as described in the user manual.
d) Precipitates in cartridges and buffers	Make sure the extraction room is not too cold; temperatures at ~23/24°C may help to prevent precipitation. Do a careful visual inspection of the cartridges. Warm EZ1 cartridges. Have buffer MTL pre-warmed just prior to addition to sample lysate and also check for crystallization. Do not warm whole MTL bottle, but pre-warm the required aliquots at 56°C. Have sample lysate hot off the incubator. If excessive precipitate or gelatinous substance is observed after addition of buffer MTL, warm lysate to 56°C until no precipitate is observed. When using ATL lysis buffer, ensure the buffer is warm and no precipitates are present when creating diluted ATL.

	Comments and suggestions
DNA stability	
a) Stability of DNA stored in water	Elute in TE buffer instead of water. Elution in TE buffer gives comparable performance and provides increased stability for long-term storage of small amounts of purified DNA.
b) Loss of DNA to plastic surfaces	The use of carrier RNA can help to prevent adsorption of DNA to the surface of tubes or plates used for storage.
Downstream applications	
a) Insufficient DNA used in downstream applications	If possible, repeat the downstream application using more eluate.
b) Excess DNA used in downstream applications	Excess DNA can overload STR amplifications. Quantify the purified human DNA by real time PCR based methods.
c) Inhibition	Typically, eluates are free from any inhibitors. In rare cases (e.g., old bones) inhibitors might get carried over. If possible, lower the sample volume used for amplification, or re-purify the eluate.
Workflows	
Influence of treated swabs	Other than 4N6FLOQSwabs (cat. nos. WB100100, WB100101, WB100102) offered by QIAGEN, it was observed that workflows including the product version "4N6FLOQSwabs Crime Scene" which are not offered by QIAGEN might need to be reviewed. Due to an antimicrobial coating of the "4N6FLOQSwabs Crime Scene", workflows including Buffer G2 might be influenced and it is recommended to consider the comparison by using Buffer ATL instead.

Ordering Information

Product	Contents	Cat. no.
EZ1&2 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers and Reagents	952034
EZ2 Fx Connect	Robotic instrument for automated purification of nucleic acids from up to 24 samples, 1-year warranty on parts and labor	9003220
Accessories		
Buffer G2 (260 mL)	Lysis buffer for EZ1 DNA procedures	1014636
DTT (1 mL)	1M DTT, forensic grade quality; for sperm cell lysis	1117316
Buffer MTL (54 mL)	–	19112
QIAGEN Proteinase K (1.2 mL)	1.2 mL (>600 mAU/mL, solution)	1014023
TissueLyser III	Universal laboratory mixer-mill disruptor	9003240
Grinding Jar Set, S. Steel (2 x 10 mL)	2 Grinding Jars (10 mL), 2 Stainless Steel Grinding Balls (20 mm)	69985
EZ2 Connect Tip Rack – Large Volume	Rack for use with EZ2 Connect; for Large-Volume Tubes	9027011
7 mL Large-Volume Tubes (48)	Two bags of 24 large-volume tubes	951954
HID-related products		
4N6FLOQSwabs in peelpouch (100)	Swabs with molded breakpoint, packaged individually in a convenient peelpouch; for sample collection	WB100100
4N6FLOQSwabs in dry tube (100)	Plastic tube guarantees swab integrity and avoids the risk of sample contamination during transport; for sample collection	WB100101
4N6FLOQSwabs with active drying system (50)	Tube ensures sample drying in the tube preventing microbial growth; for sample collection	WB100102
Investigator Lyse&Spin Basket Kit (50)	50 pouches containing 50 baskets and 100 collection tubes	19597

Product	Contents	Cat. no.
Investigator Lyse&Spin Basket Kit (250)	10 pouches containing 5 x 50 baskets and 5 x 50 collection tubes	19598
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387216
Investigator Quantiplex Pro FLX Kit (576)	For use on Applied Biosystems Real-Time Systems: 6x single blistered 96-well optical PCR plates with Master Mix, Control DNA M1, and QuantiTect Nucleic Acid Dilution Buffer	387516
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN Rotor-Gene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Investigator 24plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free Water	382415
Investigator 26plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, allelic ladder 24plex, and Nuclease-free Water	382615
Investigator ESSplex SE QS Kit (100)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free Water	381575
Investigator Argus X-12 QS Kit (25)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, Nuclease-free Water	383223
Investigator Argus Y-28 QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, allelic ladder, DNA Size Standard, Nuclease-free Water	383625

* Larger sizes are available. Please inquire.

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Document Revision History

Date	Changes
07/2023	Added protocols: 1.5 mL sample tube protocol for DNA purification (page 1); RNA/DNA co-extraction (page 54); and Bone extra-large volume protocol for DNA purification (page 1). Minor edits affected by the additional protocols. Adjusted Ordering Information. Corrected the table headings in the Document Revision History. Layout changes.
05/2024	Updated several parts of the handbook to include additional protocols for samples taken from firearms and cartridge casings. Adjusted Ordering Information. Updated the Troubleshooting Guide.
04/2025	Updated workflow diagrams for the protocols for firearms and cartridge casings samples. Updated Table 1. Added Buffer G2 to the list of required reagents for the firearms and cartridge casings protocol. Updated mentions of TissueLyser II to TissueLyser III and corresponding catalog numbers. Updated the Troubleshooting Guide.

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