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EZ2® Connect Fx Recovery Procedure Instruction Manual

For use with EZ2 DNA Investigator® Sep&Prep Kit

CE

REF 9003220

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Sample to Insight

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1. Introduction

EZ2 Connect Fx recovery mechanism is available to ensure that sample recovery is possible after an unexpected protocol execution problem. This manual describes how to perform the recovery process (both manually and semi-automatically) by using QIAamp® DNA Investigator Kit. The semi-automatic execution saves time and allows you to continue EZ2 Connect Fx process in a few steps. The manual recovery process allows you to purify your samples in the usual QIAGEN® standard, even if EZ2 Connect Fx instrument becomes inoperable.

2. 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.

- QIAamp DNA Investigator Kit (cat. no. 56504)
- Ethanol (96–100%)
- 1M DTT (cat. no. 1117316)
- Buffer G2 (cat. no. 1014636)
- Thermal mixer, heated orbital incubator, heating block, or water bath
- Microcentrifuge with rotor for 2 mL tubes

3. General Instructions

3.1. System description

Note: If the device was switched off unintentionally: Start the device. The recovery screen should appear. If it is not possible to start the device, please contact QIAGEN Technical Support.

Note: If the sample remains in the tip after the device switched off: Place a tube under the tip and remove the tip from the pipette head. The liquid will now run out of the tip. If you have any problems with this step, please contact QIAGEN Technical Support.

Before you proceed, please read these general instructions first.

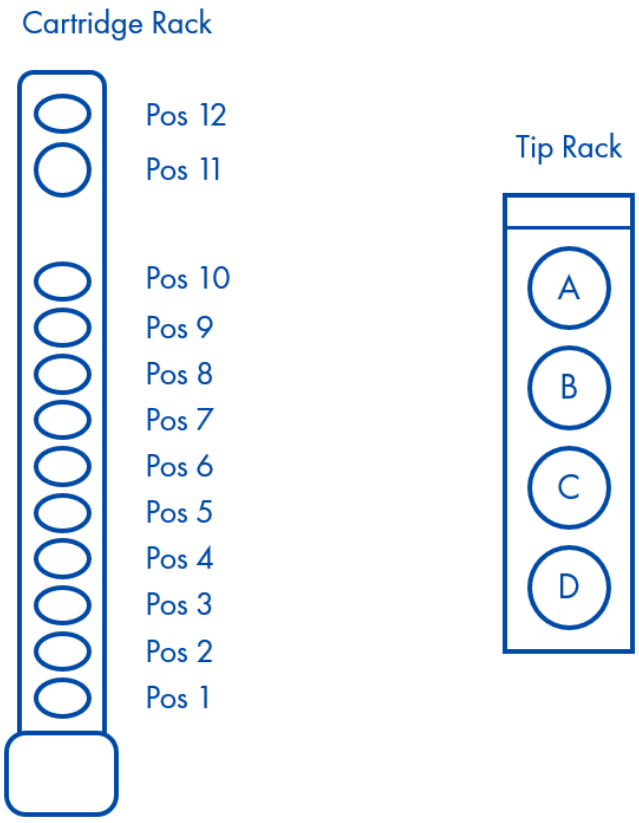

Figure 1. Position key for sample location from recovery screen description.

Figure 2. Example of a recovery screen (displayed after unintended protocol abortion).

Regardless of the respective recovery step, the following steps must be performed first:

1. The display message shows important information. Note the position of the sample and the step to be performed in the manual.
2. Open the hood.
3. Remove and keep the tubes/cartridges containing the sample.

   Important: Label the sample tubes/cartridges and be careful not to mix up their order.
4. Proceed with the indicated manual recovery step using the table in Section 4.

4. Index for Recovery Process

Indicated step	Manual recovery	Semi-automatic recovery	Process status
1	5.1	6.1	Sample untouched / NSF removal
2	5.2	6.2	During pellet wash
3	5.3	6.3	Buffer MTL added to sample
4	5.3	6.4	During bead preparation
5	5.4	6.5	During 1st binding step
6	5.4	6.6	During wash 1
6	5.4	6.6	During wash 2
6	5.4	6.6	During wash 3
6	5.4	6.6	During wash 4
6	5.4	6.6	During 2nd binding step
7	6.7	6.7	During elution step 1
8	6.8	6.8	During elution step 2
9	6.9	6.9	During elution step 3
10	6.10	6.10	After magnetic separation

5. Manual Sample Recovery and Processing Procedure

5.1. Manual recovery from unprocessed sample lysates

1. Centrifuge the D-Tube for 5 min at 15,000 rpm (23,390 x *g*) to pelletize the sample again.
2. Carefully remove the supernatant without disturbing the pellet to a new 2 mL sample tube. This is the non-sperm fraction (NSF).
3. Continue with "5.5 Manual recovery".

The NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

5.2. Recovery of samples during pellet wash step

1. Centrifuge the D-Tube for 5 min at 15,000 rpm (23,390 x *g*) to pelletize the sample again.
2. Carefully discard the supernatant without disturbing the pellet.
3. Continue with "5.5 Manual recovery".

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

5.3. Recovery of samples during resuspension step

1. Transfer the MTL buffer containing the sample from cartridge rack well 1 to a new 2 mL sample tube.

   Note: To simplify pipetting, it is recommended to further open the already pierced cartridge well with a tip.
2. Resuspend and add the beads from well 2 of the cartridge to the new sample tube.
3. For binding, place the sample in a shaker for 5 min at 1200 rpm.
4. Separate magnetic beads by centrifugation at 1200 rpm, or by using a magnetic stand.
5. Carefully remove the supernatant without disturbing the bead pellet.
6. Continue with "5.5 Manual recovery".

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

5.4. Recovery of samples during binding and wash steps

1. Resuspend the beads inside the respective cartridge well and the elution tube by pipetting up and down.

   Note: To simplify pipetting, it is recommended to further open the already pierced cartridge well with a tip.

2. Transfer both solutions into a new 2 mL sample tube.
3. Separate magnetic beads by centrifugation, or by using a magnetic stand.
4. Carefully remove the supernatant without disturbing the bead pellet.
5. Continue with "5.5 Manual recovery".

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

5.5. Manual recovery

1. Prepare the lysis master mix for each sample:
   - 1M DTT: 35 µL
   - Buffer G2: 355 µL
   - Proteinase K: 10 µL
2. Add lysis master mix to the sample and place the 2 mL tube in a thermal mixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.
3. Briefly centrifuge the 2 mL tube to remove drops from the inside of the lid.
4. Add 200 µL Buffer AL to the sample lysate. Add 100 µL EtOH, close the lid, and mix by pulse-vortexing for 10 s.
5. Carefully transfer the 700 µL sample to a QIAamp MinElute® column (in a 2 mL collection tube) without wetting the rim.
6. Centrifuge the samples at 6000 x *g* for 1 min.
7. Place the QIAamp MinElute column in a clean 2 mL collection tube and discard the collection tube containing the flow-through.
8. Add 500 µL Buffer AW1 from the QIAamp DNA Investigator Kit to the QIAamp MinElute column.
9. Close the lid and centrifuge at 6000 x *g* for 1 min.
10. Carefully discard the flow-through from the collection tube then place the QIAamp MinElute column back into the collection tube.
11. Add 700 µL buffer AW2 from the QIAamp DNA Investigator Kit to the QIAamp MinElute column.
12. Close the lid and centrifuge at 6000 x *g* for 1 min.
13. Carefully discard the flow-through from the collection tube and place the QIAamp MinElute column back into the collection tube.
14. Add 700 µL ethanol (96–100%) to the QIAamp MinElute column.
15. Close the lid and centrifuge at 6000 x *g* for 1 min.

16. Carefully discard the flow-through from the collection tube then place the QIAamp MinElute column back into the collection tube.
17. Centrifuge at full speed (20,000 x *g*) for 3 min to dry the membrane completely.

    Note: This step is necessary because ethanol carryover into the eluate may interfere with some downstream applications.
18. Place the QIAamp MinElute column in a clean 1.5 mL microcentrifuge tube (not provided), and discard the collection tube containing the flow-through. Carefully open the lid of the QIAamp MinElute column, and incubate at room temperature (15–25°C) for 10 min or at 56°C for 3 min.
19. Add Elution Buffer ATE (20–200 µL) to the QIAamp MinElute column.
20. Close the lid and incubate in a thermal mixer or heated orbital incubator at 56°C, with shaking at 900 rpm for 5 min.
21. Centrifuge at full speed (20,000 x *g*) for 1 min.

Sample is ready for downstream application.

Important: The manual recovery steps only ensure that the male DNA is recovered. The depletion of female DNA is no longer guaranteed, which may lead to a higher female carryover and thus could result in a mixed profile.

6. Semi-Automated Recovery Procedure

6.1. Recovery from unprocessed sample lysates

1. If liquid is present in the 1.5 mL Elution Tube from rack position D, transfer the liquid into the D-Tube.
2. Centrifuge the D-Tube for 5 min at 15,000 rpm (23,390 x *g*) to pelletize the sample again.
3. Place the D-Tube back to the respective position.
4. Replace tips, tip holders, and the Elution Tube with new labware.
5. Restart the run.

6.2. Recovery of samples during pellet wash step

1. Centrifuge the D-Tube from well 11 of the reagent cartridge for 5 min at 15,000 rpm (23,390 x *g*) to pelletize the sample again.

   The liquid inside the Elution Tube from rack position D does not contain sample and can be discarded.

   The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.
2. Place the D-Tube back into well 11 of the cartridge.
3. Replace tips, tip holders, and the Elution Tube with new labware.
4. Restart the run.

6.3. Recovery of samples during resuspension step

1. If liquid is present in the D-Tube, resuspend the pellet manually by pipetting up and down until no pellet is visible, and then transfer the liquid into well 1 of the cartridge.

   Note: To simplify pipetting, it is recommended to further open the already pierced cartridge well with a tip.
2. Place the D-Tube back into well 11 of the reagent cartridge.
3. If liquid is present in Elution Tube, transfer the liquid into well 1 of the cartridge.

   The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.
4. Replace tips, tip holders, and the Elution Tube with new labware.
5. Restart the run.

6.4. Recovery of samples during bead preparation step

1. If present, recover all beads from the 1.5 mL Elution Tube from rack position D and transfer the beads into well 2 of the cartridge.

   Note: To simplify pipetting, it is recommended to further open the already pierced cartridge well with a tip.

   The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.
2. Replace tips, tip holders, and the Elution Tube with new labware.
3. Restart the run.

6.5. Recovery of samples during 1st binding step

1. If liquid is present in the Elution Tube from rack position D, transfer liquid including the beads into well 1 of the cartridge.

   Note: To simplify pipetting, it is recommended to further open the already pierced cartridge well with a tip.

   The 2 mL Sample Tube containing the NSF can be stored for 6 months at 4°C or –20°C or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.
2. Replace tips, tip holders, and the Elution Tube with new labware.
3. Restart the run.

6.6. Recovery of samples during wash and 2nd binding step

1. Resuspend the beads inside the respective cartridge well and the Elution Tube from rack position D by pipetting up and down.

   Note: To simplify pipetting, it is recommended to further open the already pierced cartridge well with a tip.
2. Transfer both solutions into a new 2 mL unskirted microcentrifuge tube.
3. Separate magnetic beads by centrifugation, or by using a magnetic stand.
4. Carefully discard the supernatant without disturbing the bead pellet.
5. Prepare lysis master mix for each sample.
   - 1M DTT: 35 µL (70 mM)
   - Buffer G2: 455 µL
   - Proteinase K: 10 µL
6. Add lysis master mix to the sample and place the 2 mL tube in a thermal mixer or heated orbital incubator, and then incubate at 56°C with shaking at 900 rpm for 1 h.
7. Briefly centrifuge the 2 mL tube to remove drops from the inside of the lid.
8. Process the samples by using the EZ1&2 DNA Investigator Kit using the Large-Volume or Large-Volume RT Protocol.

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

Note: A higher amount of DNA from the female donor may be present.

6.7. Recovery of samples during elution step 1

1. Recover all beads and the entire Elution Mix and transfer it into the 1.5 mL Elution Tube from rack position D.
2. Continue lysis in the heater at 60°C for 10 min at 900 rpm.
3. Start inactivation at 95°C for 15 min at 900 rpm.
4. Briefly centrifuge the 1.5 mL tube to remove drops from the inside of the lid.
5. Separate magnetic beads by centrifugation, or by using a magnetic stand.
6. Transfer the supernatant to a new 1.5 mL Elution Tube.

   Note: A higher elution volume may be present.

The Sample containing the sperm-fraction is ready for downstream applications (qPCR, CE-STR, NGS).

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

6.8. Recovery of samples during elution step 2

1. Recover all beads and the Elution Mix and transfer it into the 1.5 mL Elution Tube from rack position D.
2. Start inactivation at 95°C for 15 min at 900 rpm.
3. Briefly centrifuge the 1.5 mL tube to remove drops from the inside of the lid.
4. Separate magnetic beads by centrifugation, or by using a magnetic stand.
5. Transfer the supernatant to a new 1.5 mL Elution Tube.

   Note: A higher elution volume may be present

The sample containing the sperm-fraction is ready for downstream application (qPCR, CE-STR, NGS).

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

6.9. Recovery of samples in the elution step 3

1. Recover all beads and the entire Elution Mix and transfer it into the 1.5 mL Elution Tube from rack position D.
2. Place samples in a shaker for 5 min at 900 rpm.
3. Separate magnetic beads by centrifugation or by using a magnetic stand.
4. Transfer the supernatant to a new 1.5 mL Elution Tube.

   The sample containing the sperm-fraction is ready for downstream application (qPCR, CE-STR, NGS).

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

6.10. Recovery after magnetic separation

1. Recover 1500 µL of beads and sample from the tip holder by using the EZ2 tip that is already present in the tip holder.

   Note: Not all pipettes fit the EZ2 tips.

2. Separate magnetic beads by centrifugation or by using a magnetic stand.
3. Carefully discard as much supernatant as possible without disturbing the bead pellet.
4. Prepare lysis master mix for each sample:
   - 1M DTT: 35 µL (70 mM)
   - Buffer G2: 455 µL
   - Proteinase K: 10 µL
5. Add lysis master mix to the sample and place the 2 mL tube in a thermal mixer or heated orbital incubator, and incubate at 56°C with shaking at 900 rpm for 1 h.
6. Briefly centrifuge the 2 mL tube to remove drops from the inside of the lid.
7. Process the samples by using the EZ1&2 DNA Investigator Kit using the Large-Volume or Large-Volume RT protocol.

The 2 mL Sample Tube containing the NSF can be stored for 4 weeks at 2–8°C or for 6 months at –20°C, or directly processed with the EZ1&2 DNA Investigator Kit using the Large-Volume Protocol.

Note: A higher female yield is to be expected here since the magnetic beads and the sample were in the waste of the pellet wash.

Document Revision History

Date	Description
08/2025	Initial release

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