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EZ2® Connect Fx Recovery Procedure Instruction Manual

For use with EZ1&2 DNA Investigator® Kit

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Sample to Insight

QIAGEN

Contents

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) with the QIAamp DNA Investigator Kit. The semi-automatic execution is very time saving and allows you to continue with ease the 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 the EZ2 Connect Fx instrument is defective.

2 Equipment and reagents to be supplied by user (for manual recovery)

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%)*
- Thermomixer, heated orbital incubator, heating block or water bath
- Microcentrifuge with rotor for 2 ml tubes

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

3 General Instructions

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 the 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 this general instructions first.

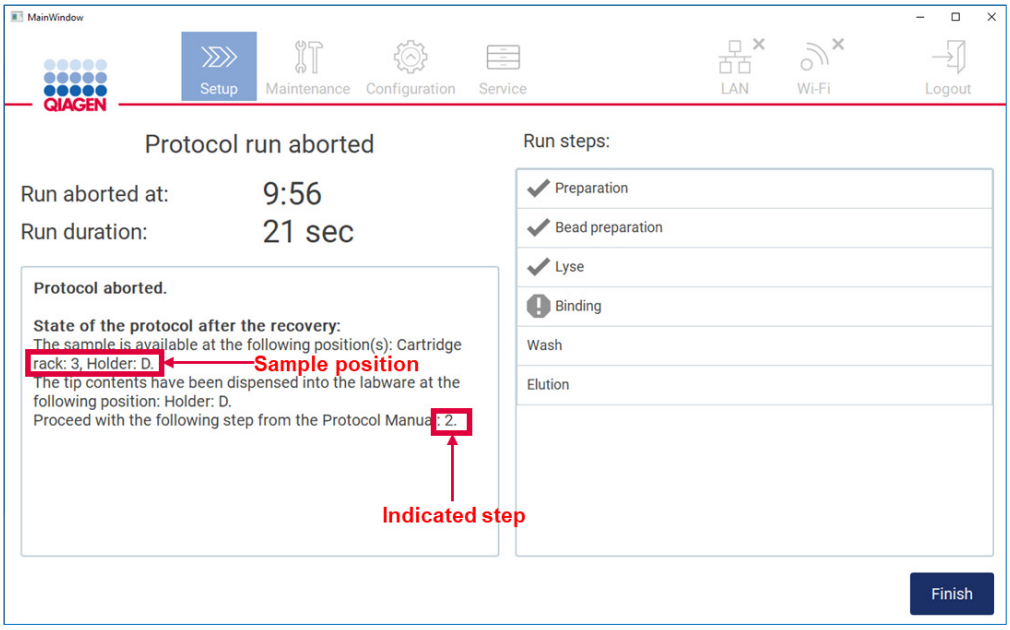

Figure 1: Recovery Screen (displayed after unintended protocol abortion).

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

1. The display message shows an 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 sample containing tubes/cartridges.
   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 Step	Semi-automatic Recovery step	Process status
1	5.1	6.1	Sample untouched
2	5.2	6.2	Buffer MTL added to the sample, beads may or may not have been added
3	5.3	6.3	During wash 1
4	5.4	6.4	During wash 2
5	5.5	6.5	During wash 3
6	5.6	6.6	During rinse step
7	5.7	6.7	During elution step

5 Manual Sample Recovery and Processing Procedure

5.1 Manual Recovery from unprocessed sample lysates (Step 1)

1. *a.)* **Recovery of Trace, Trace TD, Normalization, or Normalization TD protocols:**
   Add 200 µl buffer AL to the sample lysate. Add 100 µl EtOH, close the lid, and mix by pulse-vortexing for 10 sec.

   b.) **Recovery of Large Volume protocols with 400 µl buffer MTL added to the sample tube:**
   No addition of buffer AL required. Add 250 µl EtOH, close the lid, and mix by pulse-vortexing for 10 sec.

   c.) **Recovery of Large Volume protocols with no buffer MTL added:**
   Add 500 µl buffer AL to the sample lysate. Add 250 µl EtOH, close the lid, and mix by pulse-vortexing for 10 sec.
2. Carefully transfer the sample to a QIAamp MinElute® column (in a 2 ml collection tube) without wetting the rim. Large Volume recovery requires to load the sample in two steps, with a maximum of 700 µl applied to the column.
3. Centrifuge the samples at 6000 x g for 1 min.
4. Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.
5. Add 500 µl Buffer AW1 from the QIAamp DNA Investigator Kit to the QIAamp MinElute column and continue with Step 2 of **Manual Recovery 2**.

5.2 Manual Recovery 1 (Step 2)

1. Carefully transfer 700 µl lysate from the recovered sample to a QIAamp MinElute® column (in a 2 ml collection tube), without wetting the rim.
2. Close the lid and centrifuge at 6000 x g for 1 min.
3. Carefully discard the flow-through from the collection tube then place the QIAamp MinElute column back into the collection tube.
4. Carefully apply the remaining lysate to the QIAamp MinElute column without wetting the rim.
5. Close the lid and centrifuge at 6000 x g for 1 min.

   Note: To save the lysates from Large Volume protocols, a third loading step might be required.

   Note: Ensure the entire lysate, including magnetic particles, has been transferred to the QIAamp MinElute column.

6. Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.
7. Add 500 µl Buffer AW1 from the QIAamp DNA Investigator Kit to the QIAamp MinElute column and continue with Step 2 of **Manual Recovery 2**.

5.3 Manual Recovery 2 (Step 3)

1. Carefully transfer 700 µl sample, including all magnetic particles to a QIAamp MinElute column (in a 2 ml collection tube), without wetting the rim.
2. Close the lid and centrifuge at 6000 x g for 1 min.
3. Carefully discard the flow-through from the collection tube and place the QIAamp MinElute column back into the collection tube.
4. Add 700 µl buffer AW2 from the QIAamp DNA Investigator Kit to the QIAamp MinElute column and continue with Step 2 of **Manual Recovery 3**.

5.4 Manual Recovery 3 (Step 4)

1. Carefully transfer 700 µl sample, including all magnetic particles to a QIAamp MinElute column (in a 2 ml collection tube), without wetting the rim.
2. Close the lid and centrifuge at 6000 x g for 1 min.
3. Carefully discard the flow-through from the collection tube and place the QIAamp MinElute column back into the collection tube.
4. Add 700 µl ethanol (96–100%) to the QIAamp MinElute column and continue with Step 2 of **Manual Recovery 4**.

5.5 Manual Recovery 4 (Step 5)

1. Carefully transfer 700 µl sample, including all magnetic particles to a QIAamp MinElute column (in a 2 ml collection tube), without wetting the rim.
2. Close the lid and centrifuge at 6000 x g for 1 min.
3. Carefully discard the flow-through from the collection tube and place the QIAamp MinElute column back into the collection tube.
4. Centrifuge at full speed (20,000 x g) for 3 min. to dry the membrane completely.
5. Carefully open the lid of the QIAamp MinElute column and incubate at 56°C for 3 min.
6. Place the QIAamp MinElute column in a clean 1.5 ml microcentrifuge tube (not provided; without lid), and discard the collection tube containing the flow-through.

7. Add Elution Buffer ATE (i.e., 20–200 µl) to the QIAamp MinElute column.
8. Close the lid and incubate in a thermomixer or heated orbital incubator at 56°C, with shaking at 900 rpm for 5 min. Centrifuge at full speed (20,000 x g) for 1 min.

5.6 Manual Recovery from rinse step (Step 6)

1. Split the 1200 µl rinse volume and beads into two 600 µl. Add 600 µl buffer AL and 300 µl EtOH to each. Close the lid, and mix by pulse-vortexing for 10 sec.
2. Carefully transfer 750 µl sample to QIAamp MinElute® columns (in a 2 ml collection tube), without wetting the rim.
3. Centrifuge at 6000 x g for 1 min.
4. Repeat Steps 2 and 3 until the entire lysate of both tubes is loaded onto the QIAamp MinElute® column.
5. Place the QIAamp MinElute column in a clean 2 ml collection tube and discard the collection tube containing the flow-through.
6. Add 500 µl Buffer AW1 from the QIAamp DNA Investigator Kit to the QIAamp MinElute column, and continue with Step 2 of **Manual Recovery 2**.

5.7 Recovery of Samples in the elution step (Step 7)

1. Place samples in a shaker for 5 min at 900 rpm.
2. Separate magnetic beads by centrifugation, or by using a magnetic stand.
3. Transfer the eluate to a new tube.

6 Semi-automated Recovery Procedure

6.1 Recovery from unprocessed sample lysates (Step 1)

1. Add new labware.
2. Restart the run.

6.2 Recovery of samples in binding step (Step 2)

Trace or Trace Tip Dance protocols:

1. Recover the lysate and all beads, if already added. The expected volume is approximately 900 µl.
2. Use as samples in the Large Volume protocol (old version, with no MTL from well 10).

Large Volume protocols (new protocols using MTL from well 10):

1. Recover the lysate and all beads, if already added. The maximum expected volume is approximately 1600 µl. Split into two fractions of maximum 900 µl.
2. Use as samples in a Large Volume protocol (old version, with no MTL from well 10).

Normalization, or Normalization Tip Dance protocols:

1. No normalized recovery is possible. In case no sample material is available to re-run the normalization protocols, the recovery procedure for Trace and Trace Tip Dance protocols can be applied.

6.3 Recovery of samples in wash 1–3 (Steps 3–5)

1. Carefully discard the supernatant of the wash buffer in the Cartridge (beads should be covered with washing buffer).
2. Re-suspend the beads by pipetting up and down.
3. Transfer the remaining beads from the Cartridge into the elution tube.
4. To get a bead pellet, centrifuge the elution tube at 6000 x g for 1 min.
5. Carefully discard approximately 200 µL wash buffer from the elution tube.
6. Recover all beads in a volume of 200 µL of the corresponding wash buffer.
7. Use sample in a Trace protocol.

6.4 Recovery of samples in the rinse step (Step 6)

1. Recover all beads and the entire water used for the rinse.
2. Split into two fractions of approximately 500 µl and use as samples in a Large Volume protocol. Pool eluates of split samples after the run.

6.5 Recovery of samples in the elution step (Step 7)

1. Place samples in a shaker for 5 min. at 900 rpm.
2. Separate magnetic beads by centrifugation, or by using a magnetic stand.
3. Transfer the eluate to a new tube.

Document Revision History

Date	Changes
11/2021	Initial draft of the EZ2® Connect Fx Recovery Procedure Instruction Manual.

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