



Quick-Start Protocol

April 2026

EZ2[®] DNA Investigator[®] Sep&Prep Kit

For automated processing of sexual assault samples and sperm cell DNA preparation using the EZ2 Connect Fx instrument

The EZ2 DNA Investigator Sep&Prep Kit (cat. no. 952134) can be stored at room temperature (15–25°C). The Elution Buffer should be stored at 2–8°C. Do not freeze the reagent cartridges. Reagent cartridge and Reagent UI must be stored protected from the light. Under these conditions, the components are stable until the expiration date indicated on the kit.

Further information

- *EZ2 DNA Investigator Sep&Prep Handbook*: www.qiagen.com/HB-2432
- *EZ2 Connect and EZ2 Connect Fx User Manual*: www.qiagen.com/HB-2908
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- This protocol is for solid sample materials only (e.g., swabs).
- The buffer in well 1 (nearest to the front of the EZ2 Connect Fx when the reagent cartridge is loaded) and well 3 of the reagent cartridge may form precipitates upon storage. If necessary, redissolve by mild agitation at 37°C and then place at room temperature.
- Before loading the reagent cartridges into the EZ2 Connect Fx, invert them 4 times to mix the magnetic particles, and then tap to deposit the reagents at the bottom of their wells. Check that the magnetic particles are completely resuspended.

Procedure

1. Place the sample material into a D-Tube.
2. Prepare the lysis mix (475 µL Lysis Buffer + 25 µL Proteinase K), add 500 µL to the sample, and incubate in a thermal shaker at 56°C for 1 h set at 900 rpm. Briefly centrifuge to remove drops from inside the cap.
3. Open the D-tube. Carefully cut off the lid from the lower hinge. Cut the hinge as close to the reaction vessel as possible.

4. Transfer the material with a tweezer into a QIAshredder column, close the QIAshredder, and place the basket back into the same D-Tube. Centrifuge the D-Tube for 5 min at $21,000 \times g$ (15,000 rpm).
5. Remove the QIAshredder from the D-Tube without disturbing the pellet.
6. Prepare the EZ2 Connect Fx and load the EZ2 DNA Investigator Sep&Prep reagent cartridges.
7. Prepare the elution mix (132 μ L Elution Buffer, 5 μ L of 1:10 diluted Proteinase K, 4.5 μ L of 1:100 diluted Reagent UI, and 78.5 μ L Nuclease-free Water) and aliquot 220 μ L into each 1.5 mL microtube.
8. Prepare the Standard Tip Rack.
 - Position A: open 2 mL sample tube
 - Position B: open 1.5 mL microtube with 220 μ L elution mix
 - Position C: Tip holder with inserted tip
 - Position D: open 1.5 mL elution tubeThe 1.5 mL elution tube will contain the sperm fraction and the 2 mL sample tube will contain the non-sperm fraction at the end of the run.
9. Load the D-Tube with sample into well 11 of the Sep&Prep reagent cartridge. Do not disturb the pellet in the D-Tube. Press the D-Tube completely down into the well.
10. Load the rack into the EZ2 Connect Fx instrument. Start the run.

After the run, the sperm fraction is ready for immediate downstream application without further purification. The non-sperm fraction can be stored at 4–8°C for 4 weeks or at –20°C for 6 months, or can be purified using the EZ1&2 DNA Investigator Kit from rack position A.

Document Revision History

Date	Description
08/2025	Initial release
04/2026	Added protocol step to cut lid from D-Tube. Revised protocol step for preparation of the Elution Mix.



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