

EZ2 DNA Investigator Sep&Prep Kit

Automated processing of sexual assault samples using EZ2® Connect Fx

1 in 3 women experience physical or sexual violence in their lifetime¹. This staggering reality places a growing burden on forensic laboratories, where traditional differential extraction methods are often limited by sample quality or the number of samples submitted. In many cases, inefficient separation of sperm cells from the non-sperm cells, especially when female cells greatly outnumber male ones, compromises downstream DNA analysis.

Extensive manual steps, such as multiple wash and centrifugation cycles, further slow down the workflow and introduce the risk of error. Our new EZ2 DNA Investigator® Sep&Prep Kit streamlines the process with a fully automated solution. It enables the separation of sperm cells from non-sperm cells and the preparation of the sperm-derived DNA for direct use in downstream applications, such as qPCR, STR or NGS.

Key features:

- Automated sexual assault sample processing of 1–24 samples per EZ2 Connect Fx run
- Improved release of sperm cells from substrate
- High recovery of sperm-derived DNA with minimal female carryover
- Reduced hands-on time compared to other standard methods
- Sperm fractions ready for downstream analysis and compatible with commercial kits (qPCR, STR and NGS) – no further purification needed

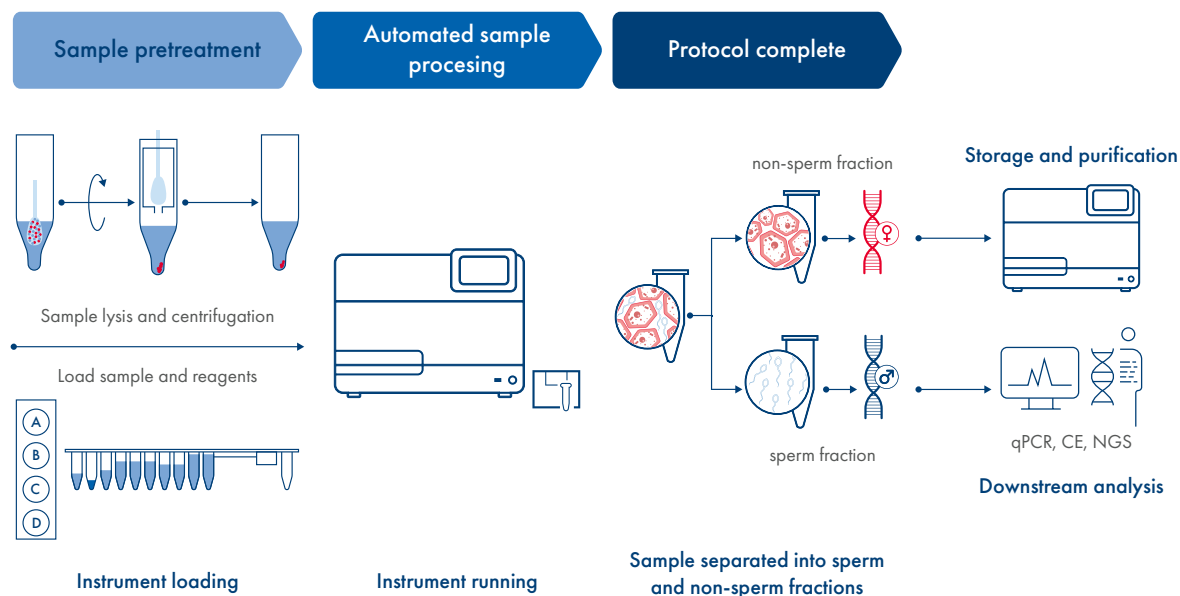


Figure 1. EZ2 DNA Investigator Sep&Prep workflow on the EZ2 Connect Fx instrument – PCR-ready extracts in <2.5 hours with minimal hands-on time.

Workflow overview

EZ2 DNA Investigator Sep&Prep Kit, together with EZ2 Connect Fx, brings true automation of sexual assault sample processing. After sample lysis and centrifugation, all remaining steps are automated on EZ2 Connect Fx, reducing hands-on time to around 20 minutes for 24 samples. The simplified and streamlined workflow minimizes the risk of processing errors and user-to-user variability, giving you greater confidence in your results compared to other differential extraction methods.

Improve the recovery of sperm-derived DNA

Obtaining a reliable autosomal STR profile is critical for the successful analysis of sexual assault samples. The two biggest challenges in this process are the recovery of sufficient sperm from the evidential material and the separation of the sperm from the victim's DNA. EZ2 DNA Investigator Sep&Prep Kit combines two new innovative features to address these issues:

- A novel buffer liberates more sperm from the evidential substrate and improves lysis and separation of the victims' cells
- A new, proprietary bead technology allows binding of the recovered sperm, enabling thorough washing and removal of the victims' DNA

With the whole process automated on EZ2 Connect Fx, the kit offers improved sperm recovery and high DNA yield, even from low-level sperm samples.

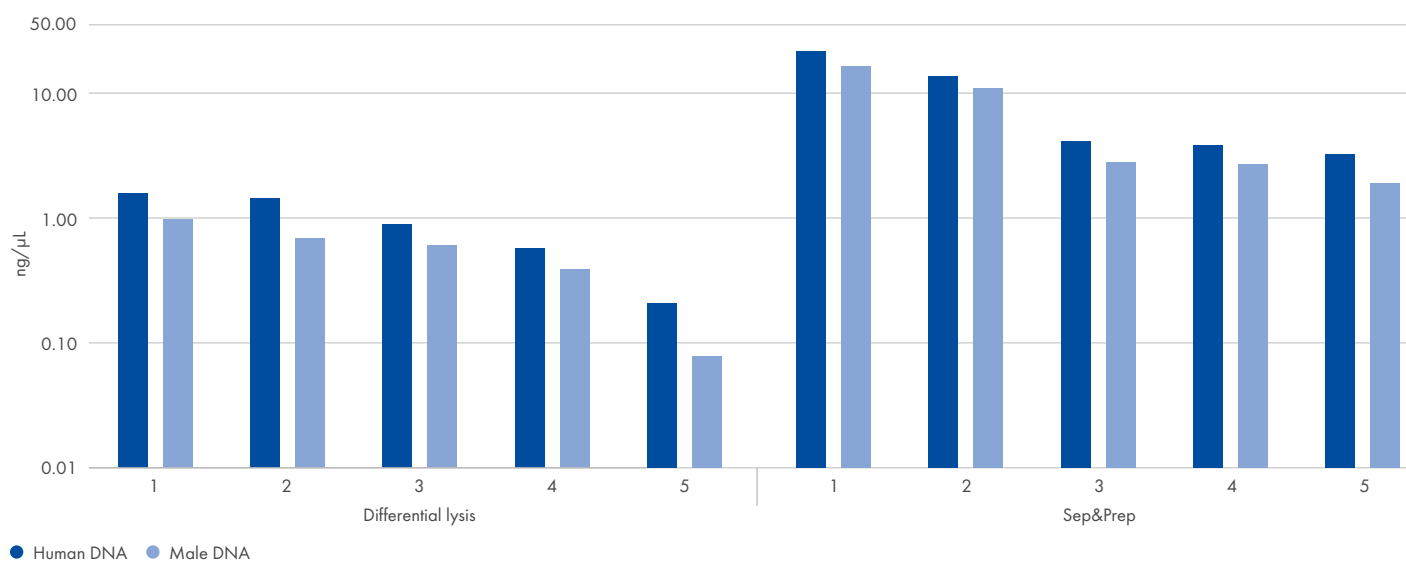


Figure 2. Comparison of DNA yields from sperm fractions.

Differential extraction and sperm-derived DNA preparation was either performed manually according to the "Pretreatment Protocol for Epithelial Cells Mixed with Sperm Cells" followed by sperm-fraction purification with the "Trace Protocol" on the EZ2 Connect Fx per the EZ1&2 DNA Investigator Kit handbook (left) or automated with the Sep&Prep workflow (right). The automated EZ2 DNA Investigator Sep&Prep method consistently outperforms the manual method across a wide range of sample input amount. Samples 1-4: Buccal swabs spiked with 25 μL (Sample 1), 12.5 μL (Sample 2), 6.25 μL (Sample 3) and 3.12 μL (Sample 4) semen; post-coital swab stored for 10 years (Sample 5); 50 μL elution volume for both methods. Quantification was done with PowerQuant® System, Promega Corporation. Data was kindly provided by the Institute of Legal Medicine Cologne, Germany.

The optimal ratio for human-to-male DNA in sperm fraction is 1:1 to ensure clear STR profile designation and accurate data interpretation. Greater ratios suggest high female contribution, which can result in mixed STR profiles that are more difficult to interpret. Compared to conventional methods, the automated Sep&Prep method yields sperm fractions with significantly lower female carryover.

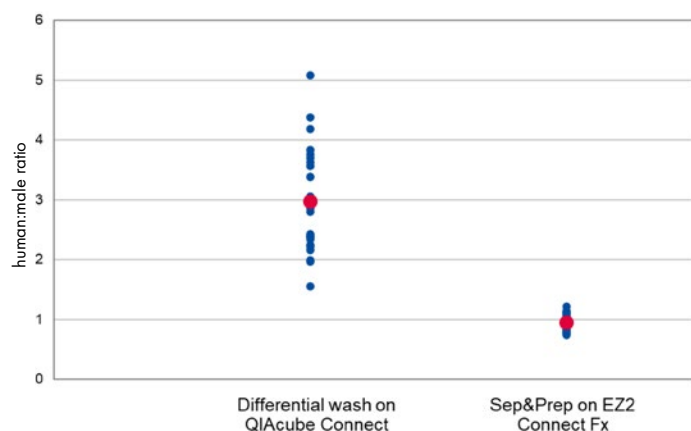


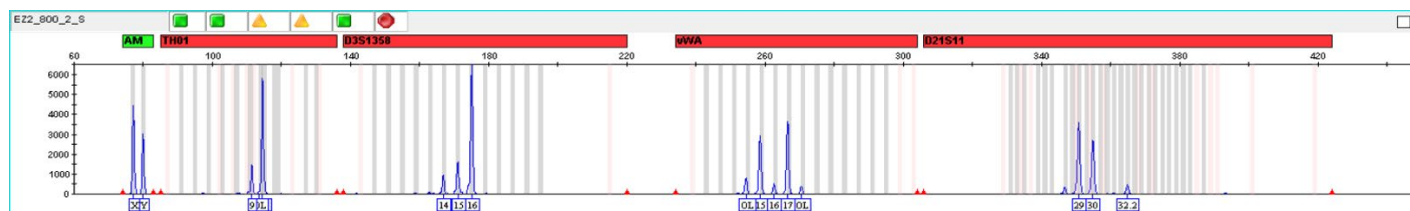
Figure 3. Ratio of human:male DNA in the sperm fractions.

Vaginal swabs from the same donor were spiked with a 1:200 semen dilution. The Sep&Prep method produced a sperm cell fraction with a 3-fold lower female contribution compared to the Differential Wash methods on QIAcube Connect. n=24 for Sep&Prep, n=23 QIAcube Connect Differential Wash; quantification done with Investigator Quantiplex Pro.

Distinct STR profiles through effective cell separation

Efficient separation of sperm and epithelial cells enhances the ability to generate distinct STR profiles from the sperm fraction. This improves the likelihood of amplifying and genotyping low-level sperm-derived DNA, ultimately increasing DNA profiling success rates and supporting successful identifications.

A EZ2 DNA Sep&Prep Kit



B QIAcube Differential Wash

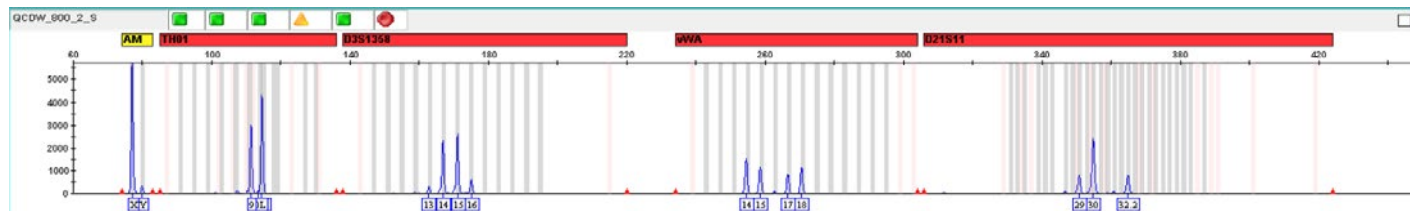


Figure 4. Example STR profile electropherograms for the sperm fractions from vaginal swabs spiked with 1:800 dilution of sperm.

A: Sample processed with EZ2 DNA Investigator Sep&Prep on EZ2 Connect Fx shows a mixed profile but with a distinct male major component. B: Sample processed with QIAcube Differential Wash protocol and purified with EZ1&2 DNA Investigator Kit, Trace protocol. Alleles cannot always be assigned to an individual person. STR profiles generated with Investigator 24plex QS Kit on the 3500 Genetic Analyzer. 6-FAM panels shown only.



Automation on EZ2 Connect Fx eliminates the high-variability introduced by manual differential lysis of post-coital samples. With minimal hands-on time – approximately 20 minutes or less – the system delivers PCR-ready sperm DNA extracts for up to 24 samples in under 2.5 hours. Analyst experience and workload no longer impact the outcome, ensuring reliable and reproducible results every time.

Figure 5. EZ2 Connect Fx instrument.

Technical Features

Features	EZ2 DNA Investigator Sep&Prep Kit
Reactions per kit	48
Sample Type	Sexual assault samples (e.g. post-coital swabs, vaginal or buccal swabs with semen, sperms mixed with different cell types, ejaculate on fabrics etc.)
Processing	Automated
Compatibility	EZ2 Connect Fx
Technology	Magnetic particles
Sample volume	500 µL
Elution Volumes	50 µL or 100 µL
Output	Ready-to-use sperm-derived DNA, NSF*†
Downstream analysis	qPCR, dPCR, STR, NGS
Time per instrument run	ca. 70 min incl. removal of NSF*, wash steps, DNA preparation, ProtK inactivation
Total time	PCR-ready extracts from sperm fraction in around 2.5 hours

*NSF = Non-sperm fraction.

†The non-sperm fraction can be stored or purified, e.g., with EZ1&2 DNA Investigator Kit (48) on the same instrument.

Ordering Information

Product	Contents	Cat. no.
EZ2 DNA Investigator Sep&Prep Kit (48)	For 48 preps on the EZ2 Connect Fx: Reagent Cartridges (Investigator Sep&Prep), Disposable Filter-Tips and Tip-Holders, Tubes, QIAshredder, Reagent UI, Lysis Buffer, Elution Buffer, Proteinase K and Nuclease-free Water	952134
EZ2 DNA Investigator Kit (48)	For 48 preps (non-sperm fraction): Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers and Reagents	952034
EZ2 Connect Fx	Robotic instrument for automated purification of nucleic acids from up to 24 samples, 1-year warranty on parts and labor	9003220

References

1. World Health Organization, Violence against women prevalence estimates, Executive summary: [9789240026681-eng.pdf](https://www.who.int/publications-detail/9789240026681-eng)



Want to learn more? Visit www.qiagen.com/sepandprep

EZ2 DNA Investigator Sep&Prep Kit meets ISO 18385 requirements.

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