November 2016

Investigator® Lyse&Spin Basket Kit Handbook

For lysis and filtration of forensic samples to remove solid sample substrates

Sample to Insight

QIAGEN

Contents

Kit Contents

Investigator Lyse&Spin Basket Kit	**(50)**	**(250)**
Catalog no.	**19597**	**19598**
Number of preps	**50**	**250**
QIAGEN Investigator Lyse&Spin Baskets	50	5x50
Collection Tubes	100	5x50
Quick-Start Protocol	1	1

Storage

The Investigator Lyse&Spin Basket Kit can be stored at room temperature (15–25°C).

Intended Use

The Investigator Lyse&Spin Basket Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Investigator Lyse&Spin Basket Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The Investigator Lyse&Spin Basket Kit is used to perform lysis and filtration of forensic samples on solid substrates. The procedure can be used for swabs, pieces of fabric or leather, paper, cigarette butts, chewing gum, small tapes or other sample types. All parts of the Investigator Lyse&Spin Basket Kit are treated with ethylene oxide post-production.

Principle and procedure

The Lyse&Spin baskets are used for pre-treatment of forensic samples in combination with manual and automated extraction kits, for example, EZ1® DNA Investigator, QIAsymphony® DNA Investigator, or QIAamp® DNA Investigator. They allow the combination of sample lysis and separation of solid sample substrates, for example, buccal or surface swabs, fabrics, cigarette butt paper and others, in one simple procedure. No sample transfer is required to obtain a cleared lysate.

The basket retains the lysis buffer during the lysis step of the forensic sample. Upon centrifugation on an ordinary benchtop centrifugation system, holes in the bottom of the basket open up and allow the sample lysate pass through. The lysate containing the nucleic acids is efficiently recovered and collected in the provided 2 ml collection tube, whereas the solid particles remain in the basket. Used baskets with solid sample substrates can optionally be stored in the second collection tube provided (only for cat. no. 19597).

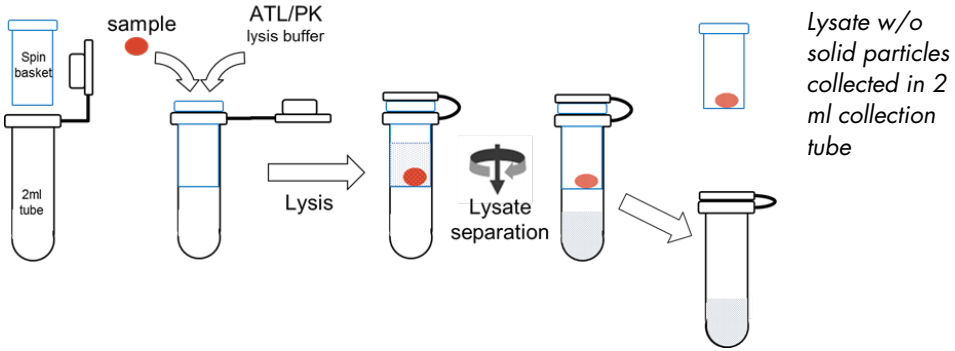

Figure 1. Investigator Lyse&Spin Basket Kit procedure.

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.

For all samples:

- Thermomixer or heated orbital incubator
- Vortexer
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)
- Lysis Buffer and proteinase K provided with the extraction kit

For sperm samples:

- 1 M dithiothreitol (DTT) for lysis of sperm cells

Important Notes

Use of Investigator Lyse&Spin Basket Kit for differential separation of sexual assault samples

The Investigator Lyse&Spin baskets are not recommended for the differential fraction separation of sexual assault samples. The baskets are not designed for a complete flow-through of sperm cells, leading to a loss of these cells in the collected lysate. QIAGEN recommends using alternative protocols for the differential lysis of sexual assault samples, for example, the differential wash protocols on the QIAcube® HID Differential Washing Station.

Protocol: Lysis and filtration of forensic samples on solid substrates

Important points before starting

- For guidance on appropriate starting material for DNA Investigator procedures, refer to the appropriate kit handbooks.
- Always wear gloves when handling the Lyse&Spin baskets to avoid contamination.

Things to do before starting

- Set a thermomixer or heated orbital incubator to 56°C.
- If Buffer ATL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- If processing semen stains, prepare an aqueous 1 M DTT stock solution. Store aliquots at –15 to –30°C. Thaw immediately before use.
- Master mixes of Buffer ATL or Buffer G2 with proteinase K (see steps 2 and 3, below) can be prepared for use on the same day to reduce pipetting steps.

Procedure

1. Place the stained material in the QIAGEN Lyse&Spin basket within a 2 ml microcentrifuge tube (provided).
2. Add 475 µl Buffer ATL (if QIAamp or QIAsymphony DNA Investigator Kits are used) or 475 µl Buffer G2 (if EZ1 DNA Investigator Kit is used)

   Note: Reduce buffer volumes to 455 µl if semen stains are processed.

   Note: Avoid pipetting on the rim of the basket. For bulky substrates, do not exceed the fill line on the basket.
3. Add 25 µl proteinase K, close the lid and mix by vortexing.

   Note: If processing semen stains, add 20 µl 1 M DTT.

4. Place the tube and basket in a thermomixer or heated orbital incubator, and incubate with shaking at 750–900 rpm at 56°C for 1 hour.

   Note: Incubation can be extended to up to 16 hours without affecting yield or quality of DNA.

   Note: If leakage of the spin basket lid is observed, the reduced shaking speed of 750 rpm is recommended.

5. Centrifuge for 1 min at a minimum of 10,000 x *g*.

   Note: Keep the lid closed during centrifugation.

   Note: Up to 20,000 x *g* can be used for centrifugation.

   Note: Make sure that no liquid remains in the basket after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane. If larger pieces of chewing gum are processed, clogging of the basket can be avoided by pressing the chewing gum against the sides of the basket.

6. Discard the basket including the solid sample substrate.

   Note: Alternatively, the basket can be stored in the second 2 ml sample tube provided (only for cat. no. 19597).

7. Continue with the extraction protocol of the flow-through fraction (see Appendix A–C, page 13).

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. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Contamination with other DNA	
a) Carryover between samples	Use a new collection tube between samples. Always wear gloves when handling QIAGEN Lyse&Spin basket.
b) Inappropriate handling	Change gloves frequently and keep tubes closed whenever possible.
Insufficient DNA recovery	
a) Incomplete liquid flow through	Extend centrifugation time or increase force up to 20,000 x *g*.
b) Incomplete lysis in the spin baskets	Incubate the forensic sample for up to 16 hours.
c) Leakage of the spin basket	Reduce shaking speed to 750 rpm. Make sure that the liquid level does not exceed the fill line on the basket.
Clogging of basket	
a) Chewing gum lysis	Push chewing gum to the wall of the basket. Some chewing gums partially dissolve in the lysis buffer and form a viscous mass. If extended centrifugation does not lead to full lysate flow through, transfer remaining lysate to the collection tube by pipetting.
b) Tape	Tapes may seal the holes of the basket during centrifugation and prevent lysate flow through. Push tape to the wall of the basket.

	Comments and suggestions
Loss of sperm cells during differential lysis of sexual assault samples	
Little or no amount of sperm cells after differential lysis of sexual assault samples	QIAGEN does not recommend the use of Lyse&Spin baskets for the differential lysis of sexual assault samples. Please use alternative protocols e.g. the differential wash protocols on the QIAcube (also see "Important Notes", page 8) Always wear gloves when handling.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Appendix A: DNA extraction using the QIAamp DNA Investigator Kit

This protocol is for isolation of total (genomic and mitochondrial) DNA from lysates obtained with the Investigator Lyse&Spin basket.

Important points before starting

- Perform all centrifugation steps at room temperature (15–25°C).

Things to do before starting

- Perform sample pretreatment according to page 9.
- Equilibrate Buffer ATE or distilled water for elution to room temperature (15–25°C).
- Set a thermomixer or heated orbital incubator to 70°C for use in step 3. If a thermomixer or heated orbital incubators are not available, heating blocks or water baths can be used instead.
- If Buffer AL contains precipitates, dissolve by heating to 70°C with gentle agitation.
- Ensure that Buffers AW1 and AW2 have been prepared according to the *QIAamp DNA Investigator Handbook*.

Procedure

1. Briefly centrifuge the 2 ml sample tube to remove drops from the inside of the lid.
2. Add 500 µl Buffer AL, close the lid, and mix by pulse-vortexing for 15 s. To ensure efficient lysis, it is essential that the sample and Buffer AL are thoroughly mixed to yield a homogeneous solution.

   Note: A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the QIAamp procedure and will dissolve during incubation in step 3.

Note: If carrier RNA is required, add 1 µg dissolved carrier RNA to 500 µl Buffer AL. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer ATE and then added to Buffer AL.

3. Place the 2 ml tube in a thermomixer or heated orbital incubator, and incubate at 70°C with shaking at 900 rpm for 10 min.

   Note: If using a thermoblock or water bath, vortex the tube for 10 s every 3 min to improve lysis.

4. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
5. Add 250 µl of ethanol (96–100%), close the lid, and mix by pulse vortexing for 15 s.

   Note: To ensure efficient binding, it is essential that the sample and ethanol are thoroughly mixed to yield a homogeneous solution.

6. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
7. Carefully transfer 700 µl lysate from step 6 to the QIAamp MinElute® column (in a 2 ml collection tube) without wetting the rim, close the lid.
8. Centrifuge at 6000 x *g* (8000 rpm) for 1 min. Carefully discard the flow-through from the collection tube and then place the QIAamp MinElute column back into the collection tube.
9. Carefully apply the remaining lysate from step 6 to the QIAamp MinElute column without wetting the rim, close the lid, and centrifuge at 6000 x *g* (8000 rpm) for 1 min.
10. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through. If the lysate has not completely passed through the membrane after centrifugation, centrifuge again at a higher speed until the QIAamp MinElute column is empty.
11. Carefully open the QIAamp MinElute column and add 500 µl Buffer AW1 without wetting the rim. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

12. Carefully open the QIAamp MinElute column and add 700 µl Buffer AW2 without wetting the rim.

13. Close the lid and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

    Note: Contact between the QIAamp MinElute column and the flow-through should be avoided. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, coming into contact with the QIAamp MinElute column. Take care when removing the QIAamp MinElute column and collection tube from the rotor, so that flow-through does not come into contact with the QIAamp MinElute column.

14. Carefully open the QIAamp MinElute column and add 700 µl of ethanol (96–100%) without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min.

15. Place the QIAamp MinElute column in a clean 2 ml collection tube, and discard the collection tube containing the flow-through.

16. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min to dry the membrane completely.

    Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

17. 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.

18. Apply 20–100 µl Buffer ATE or distilled water to the center of the membrane.

    Important: Ensure that Buffer ATE or distilled water is equilibrated to room temperature. Dispense Buffer ATE or distilled water onto the center of the membrane to ensure complete elution of bound DNA. QIAamp MinElute columns provide flexibility in the choice of elution volume. Choose a volume according to the requirements of the downstream application. Elution with small volumes increases the final DNA concentration in the eluate significantly, but reduces the overall DNA yield. Remember

that the volume of eluate will be up to 5 µl less than the volume of elution solution applied to the column.

19. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min.

    Note: Incubating the QIAamp MinElute column loaded with Buffer ATE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

Appendix B: DNA extraction on EZ1 Advanced XL

The Investigator Lyse&Spin sample collection tubes can be processed on the EZ1 Advanced XL instrument with the EZ1 Advanced XL Flip-Cap Tube Rack (cat. no. 9022818) and the EZ1 Advanced XL DNA Investigator Flip-Cap Card (cat.no. 9022763). The collection tubes should be placed into the slots of the rack and the Large-Volume protocol should be used. Alternatively, the lids of the collection tubes can be cut off and processed on BioRobot® EZ1 and EZ1 Advanced instruments with standard rack.

For automated extraction on the BioRobot EZ1, EZ1 Advanced, or EZ1 Advanced XL use the Large-Volume Protocol.

Appendix C: DNA extraction on QIAsymphony

Use Flip Cap Inserts (cat no 9244701) and the 24-sample carrier to load the Lyse&Spin collection tubes on the QIAsymphony SP. Chose Treff 96.09329.9.01 as labware.

For automated extraction on the QIAsymphony SP module use the Casework 500 µl Protocol.

Ordering Information

Product	Contents	Cat. no.
Investigator Lyse&Spin Basket Kit (50)	For 50 preps: 50 QIAGEN Lyse&Spin Baskets, 100 Collection Tubes	19597
Investigator Lyse&Spin Basket Kit (250)	For 250 preps: 5x50 QIAGEN Lyse&Spin Baskets, 5x50 Collection Tubes	19598
Accessories		
Buffer AL (264 ml)	264 ml Lysis Buffer	19075
Buffer G2 (260 ml)	260 ml Lysis Buffer	1014636
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer for 1000 preps	19076
Proteinase K (2ml)	Protease digestion during DNA and RNA preparation	19131
Related products		
QIAamp DNA Investigator Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56504
QIAsymphony DNA Investigator Kit – for automated purification of DNA from 1–96 samples		
QIAsymphony DNA Investigator Kit (192)	For 192 preps of 200 µl each from casework and reference samples: includes 2 reagent cartridges and enzyme racks and accessories	931436
Flip Cap Inserts	Flip Cap Inserts for use with the QIAsymphony SP	9244701

Product	Contents	Cat. no.
EZ1 DNA Investigator Kit – for easy, automated purification of DNA from a wide variety of forensic and human-identity samples		
EZ1 DNA Investigator Kit (48)	For 48 preps on EZ1 workstations	952034
EZ1 Advanced XL DNA Investigator Flip-Cap Card	Preprogrammed card for purification of DNA using the EZ1 Advanced XL	9018699
EZ1 Advanced XL DNA Investigator Flip cap tube rack	Tube rack for the use of flip cap tubes on EZ1 Advanced XL	9022818

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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Notes

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