August 2022

Investigator® STAR Lyse&Prep Kit Handbook

For automated purification of DNA from forensic samples using open liquid handler platforms

Sample to Insight

Contents

Kit Contents

Investigator STAR Lyse&Prep Kit Catalog no. Number of preps	(400) 931447 400
Buffer ATL	5 x 50 ml
Buffer QSL3*	5 x 45 ml
Buffer QSW1*	5 x 50 ml
Buffer QSW2	5 x 160 ml
Bead Suspension G	5 x 15 ml
Buffer ATE	5 x 20 ml
Proteinase K	2 x 6 ml
Carrier RNA	2 x 310 µg
Q-Card†	1
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for Safety Information.
† The information encoded in the bar code on the Q-Card is needed for reagent data tracking.

Shipping and Storage

The Investigator STAR Lyse&Prep Kit is shipped at ambient temperature. All buffers and reagents can be stored at room temperature (15–25°C). Do not freeze the reagent bottles. When stored properly, the reagents are stable until the expiration date on the Q-Card. Lyophilized carrier RNA is stable until the expiration date printed on the Q-Card, when stored at room temperature.

The ready-to-use Proteinase K solution is stable for up to 1 year after delivery, when stored at room temperature.

Intended Use

The Investigator STAR Lyse&Prep Kit is intended for molecular biology applications in forensic, human identity, and paternity testing. The Investigator STAR Lyse&Prep Kit is intended to be used with open liquid handler platforms for nucleic acid purification based on magnetic bead technology. 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.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Buffers QSL3 and QSW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Quality Control

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

Functional QC testing ensures that the Investigator STAR Lyse&Prep Kit meets the high standards required by forensic scientists. The Investigator STAR Lyse&Prep Kit meets ISO 18385 requirements.

Introduction

The Investigator STAR Lyse&Prep Kit, used on an automated liquid handler, reproducibly automates purification of genomic DNA, encountered in forensic and human identity applications. Purification is efficient, and purified DNA performs well in downstream analyses, such as quantitative PCR and STR analysis.

Principle and procedure

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in modified TE buffer (Buffer ATE).

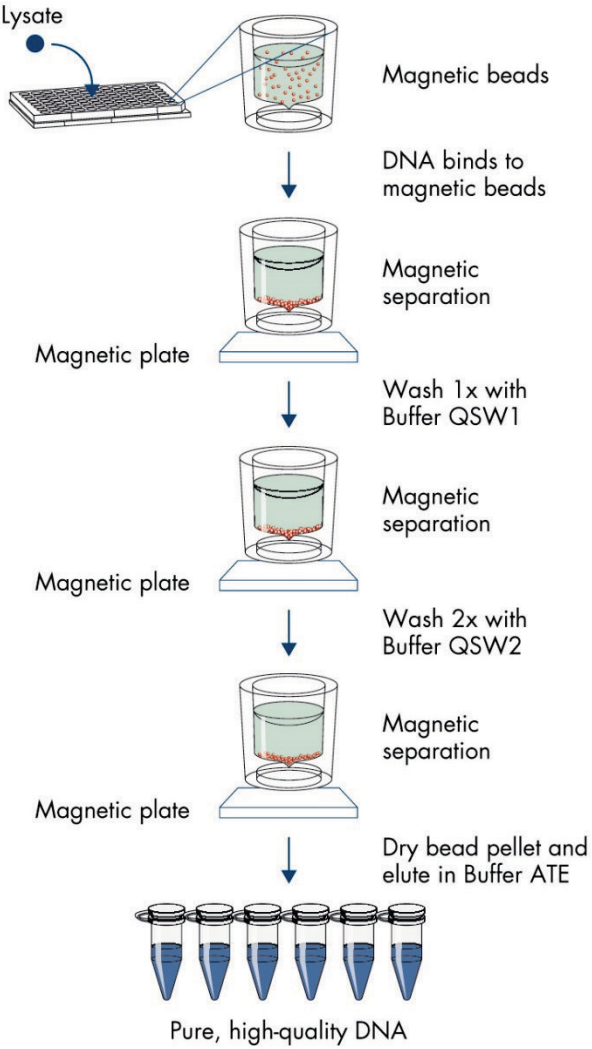

Figure 1. Workflow.

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.

All protocols

- Sample and elution labware according to Table 1
- Thermomixer
- Centrifuge
- Vortexer
- Pipets and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)

For purification of DNA from semen stains or hair

- 1 M dithiothreitol (DTT), forensic grade quality

Table 1. Labware examples for sample input and elution

Manufacturer	Type	Catalogue no.	Use
Treff*	2 ml	96.09329.9.01	Sample input/elution
Eppendorf®	1.5 ml Safe-Lock	0017 010.417-03/1109	Sample input/elution
Eppendorf	2 ml Safe-Lock	0017 010.425-03/1109	Sample input/elution
Sarstedt®	1.5 ml Safe-Lock	72.706	Sample input/elution
Sarstedt	2 ml Safe-Lock	72.695.500	Sample input/elution
Sarstedt	1.5 ml	72.690.001	Sample input/elution
Sarstedt	2 ml	72.691	Sample input/elution
VWR®	96-Well-Plate, 2.2 ml	732-0585	Sample input

* Collection tube of the Investigator Lyse&Spin Basket Kit.

Important Notes

Starting material

The amount of starting material for use in the Investigator STAR Lyse&Prep Kit procedures can vary greatly, depending on the amount of DNA in the sample. Specific guidance for starting amounts is given in the individual protocols and in Table 2.

Purification of low amounts of DNA

For purification of DNA from very small amounts of sample, such as touch DNA samples, we recommend adding carrier RNA. For samples containing larger amounts of DNA, addition of carrier RNA is optional. Add 310 µl TE buffer or water to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide into conveniently sized aliquots, and store at –30 to –15°C. Do not freeze–thaw the aliquot of carrier RNA more than 3 times. Carrier RNA should be added to the sample after the lysis is completed to avoid degradation. Carrier RNA can be added to the binding buffer (Buffer QSL3) prior to setting up the instrument run. Use the mixture of binding buffer and carrier RNA within the same day.

Precipitate in Buffer ATL

Before starting the procedure, check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70°C with gentle agitation.

Precipitate in Buffer QSL3

Buffer QSL3 may form a precipitate upon storage. If necessary, redissolve by mild agitation at 37°C and then place at room temperature (15–25°C).

Lysis with Proteinase K

The Investigator STAR Lyse&Prep Kit contains Proteinase K, which is the enzyme of choice for lysis buffers used in Investigator STAR Lyse&Prep protocols. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and is particularly suitable for short digestion times. It possesses a highly specific activity and remains stable over a wide range of temperatures and pH values, with substantially increased activity at higher temperatures. The activity of the Proteinase K solution is 600 mAU/ml solution (or 40 mAU/mg protein).

Quantification of DNA

Degraded, inhibited, or mixed DNA samples are common in forensic casework and other human identity testing applications. Such samples can create challenges in STR analysis. Prior quantification of the purified DNA, using real-time PCR is recommended and reduces the need to repeat downstream analyses. This greatly reduces costs and time, and improves the statistical relevance of results. The Investigator Quantiplex® Pro Kits use quantitative real-time PCR to quantify human total and human male DNA in a sample. These kits also detect if the sample contains inhibitors that may interfere with downstream applications, or if the DNA is degraded.

Table 2. Protocol information for different sample types

Sample type	Sample amount	Buffer ATL	Proteinase K	DTT (1 M)
Blood/saliva	Up to 50 µl	280 µl/475 µl*	20 µl/25 µl*	–
Surface swabs	1 swab	280 µl/475 µl*	20 µl/25 µl*	–
Chewing gum	Up to 40 mg	280 µl/475 µl*	20 µl/25 µl*	–
Cigarette butts	1 cm^2	280 µl/475 µl*	20 µl/25 µl*	–
Paper/similar materials	0.5–2.5 cm^2	280 µl/475 µl*	20 µl/25 µl*	–
Nail scrapings	Up to 40 mg	260 µl/455 µl*	20 µl/25 µl*	20 µl
Nail clippings	1	260 µl/455 µl*	20 µl/25 µl*	20 µl
Hair	0.5–1cm	260 µl/455 µl*	20 µl/25 µl*	20 µl
Tissues	Up to 10 mg	280 µl/475 µl*	20 µl/25 µl*	–
Blood or saliva stains	0.5 cm^2	280 µl/475 µl*	20 µl/25 µl*	–
Semen stains	0.5 cm^2	260 µl/455 µl*	20 µl/25 µl*	20 µl
Buccal swabs	1 swab	280 µl/475 µl*	20 µl/25 µl*	–

* If using the Investigator Lyse&Spin Basket Kit.

Protocol: Pretreatment for Various Casework and Reference Samples

This protocol is designed for isolation of total DNA (genomic and mitochondrial) from various types of casework and reference samples. The protocol describes the preliminary lysis using Proteinase K.

Notes before starting

- Before beginning the procedure, read "Important Notes" on page 9.
- We recommend using the Investigator Lyse&Spin Basket Kit (cat. no. 19597 or 19598) when solid sample materials have to be removed from the lysate. If using this kit, please follow the Pretreatment protocol, as outlined under "Procedure using the Investigator Lyse&Spin Basket Kit" (page 13). The Lyse&Spin Basket Kit collection tubes can be used as sample tubes.
- Heat a thermomixer to 56°C for the Proteinase K digest in step 3.

Procedure using 300 µl lysis volume

1. Place the sample into a sample tube or deep-well block.
2. Set up the Proteinase K digest according to the information provided in Table 2, page 11. Mix sample thoroughly by vortexing for 10 s.
3. Incubate at 56°C for 15 min or up to overnight in a thermomixer, shaking at 900 rpm.

   Incubation for 15 min may be sufficient to recover adequate DNA for STR typing from samples containing abundant DNA. More than 1 h is recommended where a low amount of DNA is expected. When using a deep-well block, ensure proper sealing with an adhesive tape.
4. After incubation, perform a brief centrifugation to collect liquid at the bottom of the tubes or the S-Block.

5. Transfer 300 µl lysate to a new tube or deep-well block.

   Note: Take care not to transfer any solid sample materials. See Table 1 for suitable labware.

6. Continue with automated DNA purification.

Procedure using the Investigator Lyse&Spin Basket Kit

1. Place the stained material into the Investigator Lyse&Spin Basket, inserted into a 2 ml microcentrifuge tube (provided).
2. Set up the Proteinase K digest according to the information provided in Table 2. Mix the sample thoroughly by vortexing for 10 s.

   Note: The Investigator Lyse&Spin Basket requires 500 µl lysate volume.

3. Incubate at 56°C for 1 h or up to overnight in a thermomixer, shaking at 900 rpm.
4. 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.

5. Discard the basket including the solid sample substrate.
6. Continue with automated DNA purification.

Protocol: DNA Purification

This protocol provides guidance for programming protocols for automated extraction on instruments using magnet plates to separate beads in 96 deep-well plates. The protocol parameters for 300 and 500 µl lysate volumes are outlined. Along with the general recommendations, these are provided as a good starting point for further instrument-specific optimization work.

Important points before starting

- Shaking speeds and durations stated refer to the use of a 2 mm orbital shaker and 96 DW Plates (VWR, LBCN3905-520-010). Changes to shaker specifications or plate type may require adapted speed and duration (e.g., lowering speed when using 3 mm orbital shakers to avoid cross-contamination).
- For the 500 µl protocol, splitting of lysates for binding is recommended, if 96 deep-well plates are used for processing. This will reduce cross-contamination risks. Do not reduce the binding buffer volume to lysate volume ratio, as this may affect yields from degraded samples.
- Heating during binding and elution can help to optimize yield. However, excessive heating of buffer QSL3 can cause loss of DNA during binding. We do not recommend to exceed 10 min at 50°C. First indications of this issue is the drop in D5S818 and D13S317 peak heights, independent of the STR assay used.
- Magnetic beads settle quickly. We recommend to thoroughly mix the bead suspension before any distribution step. During development, carefully check for gradients of bead pellet sizes across 96-well plates, indicating inhomogeneous bead suspension.
- Carefully remove any wash buffer after the last wash to avoid ethanol carryover. We recommend to introduce a second liquid removal step using a small tip size. Remaining ethanol can be evaporated in an air-dry step. Avoid over-drying the bead pellet, as this may lead to inefficient elution of DNA.

Table 3. Recommended protocol parameters.

Step	Parameter	300 µl Lysate Protocol	500 µl Lysate Protocol‡
Binding	Binding Buffer (µl)	720*	800†
	Bead Suspension G (µl)	100	100
	Lysate (µl)	300	500
	Shaking Speed (rpm)	1000	1000
	Shaking Time (sec)	600	600
	Shaking Temperature (°C)	50	50
Wash 1	Wash Buffer QSW1	500	500
	Shaking Speed (rpm)	1000	1000
	Shaking Time (sec)	30	30
	Shaking Temperature (°C)	30	30
Wash 2	Wash Buffer QSW2	500	500
	Shaking Speed (rpm)	1000	1000
	Shaking Time (sec)	30	30
	Shaking Temperature (°C)	30	30
Wash 3	Wash Buffer QSW2	500	500
	Shaking Speed (rpm)	1000	1000
	Shaking Time (sec)	30	30
	Shaking Temperature (°C)	30	30
Air-dry	Incubation time (sec)	600	600
Elution	Elution Buffer ATE	50–100	50–100
	Shaking Speed (rpm)	1000	1000
	Shaking Time (sec)	300	300
	Shaking Temperature (°C)	80	80

* Mixture of 360 µl QSW2 and 360 µl QSL3

† Mixture of 480 µl QSW2 and 320 µl QSL3

‡ If binding is performed in 96 deep-well plates, we recommend to split lysates to avoid cross-contamination. Use 250 µl lysate, 400 µl Binding Buffer, and 100 µl Bead Suspension. Reuse beads from the first binding step.

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 in 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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Low DNA yield		
a)	Insufficient lysis	Increase lysis time.
b)	Insufficient sample aspirated	Check sample input for remaining lysate.
c)	Insufficient amount of magnetic beads	Carefully mix bead suspension before transferring to tubes to load on the instrument.
DNA does not perform well in downstream applications		
a)	Insufficient DNA used in downstream applications	If possible, repeat the downstream application using more eluate.
b)	Excess of magnetic beads	Excess of magnetic beads may lead to insufficient drying before elution, resulting in ethanol carryover. Carefully mix bead suspension before transferring to tubes to load on the instrument.

Ordering Information

Product	Contents	Cat. no.
Investigator STAR Lyse&Prep Kit (400)	For 400 preps of 300 µl or 500 µl each from casework and reference samples	931447
Accessories		
DTT (1 ml)	1M DTT, forensic grade quality; for sperm cell lysis	1117316
HID-related products		
Investigator Lyse&Spin Basket Kit (50)	50 pouches containing 50 baskets and 100 collection tubes	19597
Investigator Lyse&Spin Basket Kit (250)	10 pouches containing 5 x 50 baskets and 5 x 50 collection tubes	19598
Investigator Quantiplex Pro Kit (200)	For use on Applied Biosystems Real-Time Systems: Quantiplex Pro Reaction Mix, Quantiplex Pro Primer Mix, Quantiplex Pro Control DNA M1, QuantiTect® Nucleic Acid Dilution Buffer	387216
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN Rotor-Gene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Investigator 24plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	382415

Product	Contents	Cat. no.
Investigator 26plex QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, allelic ladder, and Nuclease-free water	382615
Investigator ESSplex SE QS (100)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, and Nuclease-free water	381575
Investigator Argus X-12 QS Kit (25)*	Primer Mix, Fast Reaction Mix 2.0, Control DNA, allelic ladder, DNA Size Standard, Nuclease-free water	383223
Investigator Argus Y-28 QS Kit (100)*	Primer Mix, Fast Reaction Mix 3.0, Control DNA, allelic ladder, DNA Size Standard, Nuclease-free water	383625

* Larger package sizes are available.

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.

Document Revision History

Date	Changes
08/2022	Corrected the names of wash buffers in Table 3.