



September 2022

KingFisher™ Flex

Instructions for Use (Protocol Sheet)

For the preparation of KingFisher Flex Purification System using
Investigator® STAR Lyse&Prep Kit

General Information

This guide aims to aid with preparation of the KingFisher Flex purification system with QIAGEN®'s Investigator STAR Lyse&Prep Kit. It shows how to install protocols, load consumables, and perform sample purification.

The Thermo Scientific KingFisher Flex is a versatile benchtop automated extraction instrument capable of processing up to 24 or 96 samples per run. It is designed for medium- to high-throughput extraction and purification of DNA, RNA, proteins, and cells.

Important Notes

Starting material

The amount of starting material for use in 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 1.

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 (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).

Magnetic beads

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.

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 1. 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		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	0.5–1 cm	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	-
Buccal swabs	2 swabs	475 µl	25 µl	
Surface swabs	2 swabs	475 µl	25 µl	

KingFisher Flex

The instrument functions without any dispensing or aspiration parts or devices. All reagents required for processing must be dispensed prior to loading onto the instrument according to the instructions.

The KingFisher Flex is designed to work with the BindIt™ Software.

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 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.
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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).

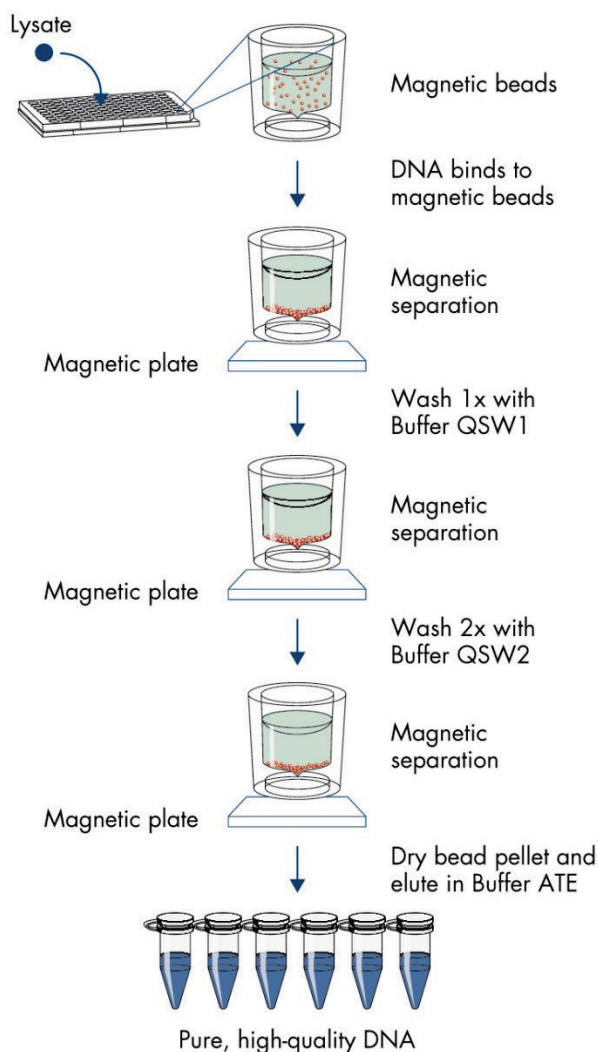


Figure 1. Workflow

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 2
- 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, cat. no. 1117316

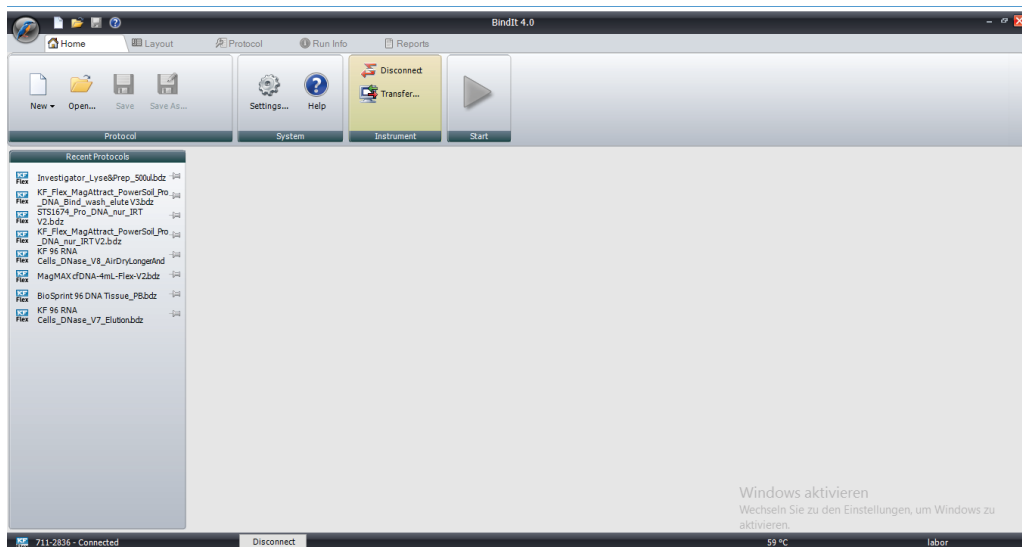
Table 2. Labware requirement for sample input and elution

Manufacturer	Type	Cat. no.	Use
Thermo Fisher	96 Deep-well plate, V bottom	95040450	Sample input/processing
Thermo Fisher	96 Tip comb	97002534	Sample processing
Thermo Fisher	96 Microplate (200 µl)	97002540	Elution

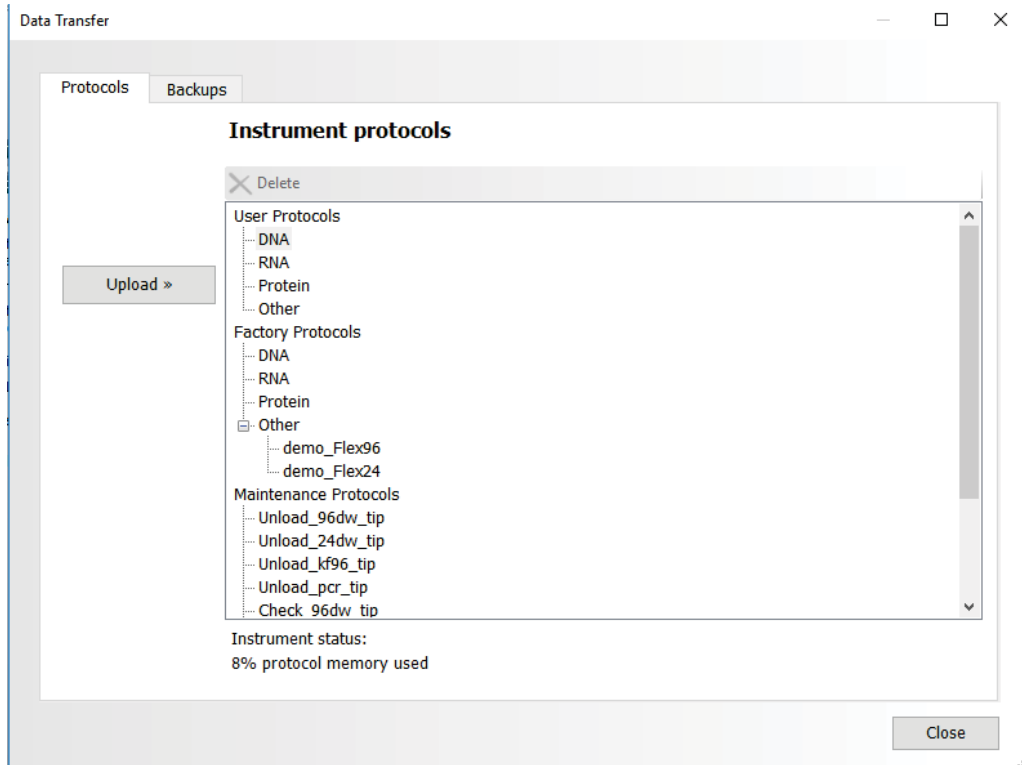
Instrument Preparation

Protocol Installation

1. Turn on the KingFisher Flex.
2. Load the BindIt software on the connect PC.
3. Within the Instrument tab, select **Transfer**.



4. Select **DNA** under User Protocols and click **Upload**.



5. Navigate to the supplied protocol and select. Name the protocol as preferred and ensure "DNA" is selected as the Category.

Send to instrument

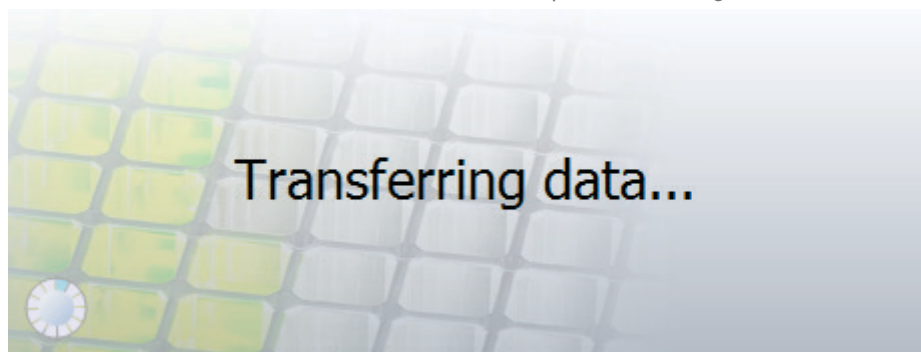
Original name: Investigator_LysePrep_500ul

Protocol name:

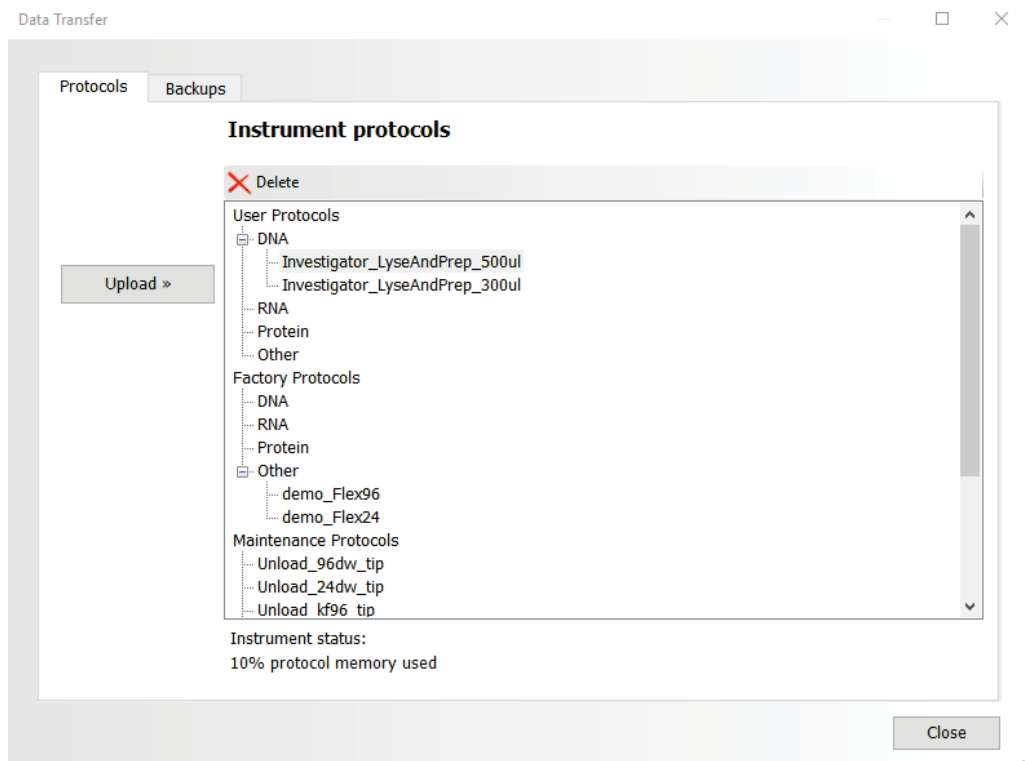
Type: User protocol

Category: DNA ▾

6. Click **OK**. The instrument will inform the user the protocol is being transferred.



7. After successful upload, the protocol will be available within the protocol menu.



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 2.
- 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”. 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 2.

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 1. 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 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.

Procedure using 500 µl lysis volume

1. Prepare deep-well plates with sample and reagents according to Table 3.
2. Either using the BindIt software or directly interfacing with the KingFisher Flex instrument, select the 500 µl Lyse&Prep protocol from the DNA menu and press **Start**.

3. Follow the on-screen instructions to correctly load the deep-well plates, tip comb, and elution plate.

Note: The KingFisher Flex has a single loading position, accessed through the sliding door and will rotate to the required position as needed.

Note: The 500 µl protocol splits the sample lysate across 2 deep-well plates to avoid overflow from the deep well plate during sample processing. Magnetic beads are only required in one plate; ensure that the plate containing magnetic beads is loaded onto the KingFisher Flex into the requested plate position “Lysate and Binding 1” as requested by the method.

4. After the run is complete, remove the elution plate and store at –20°C if not immediately required for downstream sample processing.

5. Dispose of the remaining plates and tip comb.

Table 3. Sample and reagent loading guide using a 500 µl lysate volume

Plate name	Plasticware		Reagent and volume				
Lysate and Binding 1	Deep-well plate	Sample lysate: 250 µl	Buffer QSW2: 300 µl	Buffer QSL3: 215 µl	Magnetic Beads: 80 µl	cRNA: 5 µl	Total: 850 µl
Lysate and Binding 2	Deep-well plate	Sample lysate: 250 µl	Buffer QSW2: 300 µl	Buffer QSL3: 215 µl	cRNA: 5 µl		Total: 870 µl
Wash 1	Deep-well plate	Buffer QSW1: 700 µl					Total: 700 µl
Wash 2	Deep-well plate	Buffer QSW2: 700 µl					Total: 700 µl
Wash 3	Deep-well plate	Buffer QSW2: 700 µl					Total: 700 µl
Elution	Microplate (200 µl)	Buffer ATE: 60 µl					Variable: 50 µl minimum
Tip Comb	96 Tip comb	N/A					

Procedure using 300 µl lysis volume

1. Prepare deep-well plates with sample and reagents according to Table 4.
2. Either using the BindIt software or directly interfacing with the KingFisher Flex instrument, select the 300 µl Lyse&Prep protocol from the DNA menu and press **Start**.
3. Follow the on-screen instructions to correctly load the deep-well plates, tip comb, and elution plate.

Note: The KingFisher Flex has a single loading position, accessed through the sliding door, and will rotate to the required position as needed.
4. After the run is complete, remove the elution plate and store at –20°C if not immediately required for downstream sample processing.
5. Dispose of the remaining plates and tip comb.

Table 4. Sample and reagent loading guide using a 300 µl lysate volume

Plate name	Plasticware	Reagent and volume					
Lysate and Binding	Deep-well plate	Sample lysate: 300 µl	Buffer QSW2: 300 µl	Buffer QSL3: 215 µl	Magnetic Beads: 80 µl	cRNA: 5 µl	Total: 900 µl
Wash 1	Deep-well plate	Buffer QSW1: 700 µl					Total: 700 µl
Wash 2	Deep-well plate	Buffer QSW2: 700 µl					Total: 700 µl
Wash 3	Deep-well plate	Buffer QSW2: 700 µl					Total: 700 µl
Elution	Microplate (200 µl)	Buffer ATE: 60 µl					Variable: 50 µl minimum
Tip Comb	96 Tip comb	N/A					

Revision history

Revision	Description
September 2022	Initial release

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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