ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

EliGene® Viral RNA/DNA FAST Isolation Kit
Instructions for Use

Package:

Ref. No.	Quantity
409100	100 Preps

Storage:

All kit reagents and components should be stored at room temperature (15 – 30 °C). When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label.

Intended use

The EliGene® Viral RNA / DNA FAST Isolation Kit is designed for rapid (15min) isolation of viral RNA and DNA (nucleic acids - NA) from nasopharyngeal swabs, buccal swabs, sputum and saliva using specially developed technology to remove inhibitors. The isolation is specially designed for use on low nucleic acid samples. Isolated NA shows high purity and is therefore suitable for amplification techniques such as RT-PCR of viral NA in clinical samples.

Principle of the method

The principle of isolation is based on the lysis of viral particles with the aid of detergents. In the presence of a chaotropic reagent, the nucleic acids are bound to a spin column (filter), washed and eluted in TRIS-HCl buffer without EDTA.
Isolated NA is ready for direct use in RT-PCR, RT-qPCR and other applications.

Introduction

Diagnostics of viral respiratory diseases focuses on the detection of viral nucleic acids using DNA detection methods. These methods usually require viral nucleic acids isolated from clinical material and free of reverse transcriptase and DNA polymerase inhibitors. There are several methods for isolating nucleic acids from clinical material, which can be based on the principle of paramagnetic particles, silica columns, filtration, etc. Silica column technology allows rapid removal of PCR inhibitors by easily washing the nucleic acids bound to the filter while maintaining high sensitivity to capture even small concentrations of nucleic acids.

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

Required material not included in kit

100x 1.5 ml Tube with lid for sample lysis and preparation
100x Microtube with lid used by customer for storage of isolated nucleic acids
13 ml of 96% ethanol for molecular biology

Required equipment

Microcentrifuge (12,000 x *g*)
Vortex
Microtube rack
Pipettes: 50 – 1000 µl

Kit Contents

Components	Amount (100 isolations)
Lysis Buffer Stock Solution	22 ml *+ add Solution M*
Wash Buffer 1	53 ml
Wash Buffer 2	40 ml *+ add 13 ml of 96% ethanol**
Elution Buffer	6 ml
Solution M	110 microliters
Spin Filters (Units in 2 ml Collection Tubes)	100 pcs
2 ml Collection Tubes without lids	200 pcs

**13 ml of 96% ethanol not included*

NOTES BEFORE STARTING:
Pre-preparation of solutions, before use.
For each day prepare fresh working solution of Lysis buffer by mixing 5 microliters of Solution M with 1 ml of Lysis Buffer Stock Solution. Add Solution M to the Lysis Buffer in a fume hood! In the case that you plan use all 100 preps, mix entire volume of Solution M with Lysis Buffer Stock Solution to obtain Lysis Buffer. Keep prepared Lysis Buffer at room temperature! Prepared Lysis Buffer is stable at room temperature for 24 hours.
Add 13 ml of 96% ethanol to Wash Buffer 2 before use! Use only ethanol intended for molecular biology, possibly in p.a. grade.

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Safety Data Sheets for emergency procedures in case of accidental ingestion or contact.

Reagents labelled flammable should be kept away from open flames and sparks.
Do not mix components of different lots of kits! Lysis buffer and Wash Buffer 2 are not ready to use and must be prepared!

Primary sample collection, handling and storage

Nasopharyngeal and buccal swabs:

Collect the indicated samples according to a standard protocol in sampling tubes.
Recommended swabs:
FLOQSwabs (Copan) – tampons immerse in UTM - (Universal Transport Medium, Copan)
Darcon swap - immerse swabs in transport medium for MicroTest ™ M4RT or MicroTest ™ M6 viruses (Termo Scientific)
Other sampling kits based on polymeric materials with a transport medium for viruses.
Do not use cotton swabs due to possible inhibition of the PCR reaction. The transport medium dilutes viruses and at low virus concentrations this dilution may cause false negative results.
As an alternative to virus transport medium, lysis buffer can be used, where collection swabs are immersed directly in lysis buffer diluted 1: 1 with molecular biology water without added M (Solution M). Add Solution M just before isolation according to the procedure below.
Samples should be transported to the laboratory at 4 °C (blue ice). They are stable for at least 72 hours after sampling at 4 °C. If you are not able to transport swabs to the laboratory at 4 °C, it is possible to transport samples at room temperature within six hours.
For sample storage longer than 72 hours, freeze the sample at -20 ° C.

Sputum and saliva:

Collect the indicated samples according to a standard protocol in sampling tubes. In the laboratory, dilute 1:1 sputum or saliva with PBS or physiological saline solution. In case of high viscosity, you can add another portion of PBS or physiological saline solution. Then vortex the sample thoroughly and centrifuge at 12,000 x g for 2 minutes. Then remove 200 microliters of supernatant from the top of supernatant and use for further isolation.
Sputum and saliva samples should be transported to the laboratory at 4 °C (blue ice). For sample storage longer than 48 hours, freeze the sample at -20 °C.

Detailed Isolation Protocol

It is highly recommended to read this information before you use the **EliGene® Viral RNA/DNA FAST Isolation Kit** for the first time.

Important Notes before Using

Please wear gloves at all times.

Removal of residual ethanol from the spin filter is critical for efficient elution of nucleic acids from the filter by Elution Buffer.

1. Pipette 200 µl of sample **(transport medium or diluted sputum or saliva)** into 1.5 ml tubes (not included in kit).

2. **Be sure that you have prepared fresh Lysis buffer solution mixed with Solution M (for details see Notes before starting)**.
   Add 200 µl of **Lysis Buffer** to 1.5 ml Tube with 200 µl of sample from first step, close lid and mix gently by vortexing for 10 seconds. In this step, you can add the internal control as described in the detection kit before vortexing.
   If you placed the collection swab directly into the diluted lysis buffer, use the entire volume of buffer after removing the swab and add 1 µl of Solution M, close the lid and vortex for 10 seconds. In this step, you can add an internal control before vortexing according to the instructions for the detection kit used.
   Background: Lysis Buffer is a lysis reagent containing detergent and other reagents required for complete cell and viruses lysis and binding of NA onto the silica membrane.

3. Shortly spin to collect the sample from the lid and transfer the whole liquid onto a spin filter and centrifuge at 12,000 x g for 1 minute at room temperature. Discard the flow through and reuse the collection tube.
   Background: NA binds onto the silica membrane in the spin filter because it is in a chaotropic salt condition. The liquid flow through contains unbound cell material such as denatured proteins.

4. Add 500 µl of **Wash Buffer 1** onto the spin filter. Centrifuge at 12,000 x g for 1 minute. Discard the flow through and reuse the collection tube.
   Background: Wash buffer 1 is a salt-based wash solution that cleans NA bounded onto the spin filter from impurities.

5. Add 500 µl of **Wash Buffer 2** to the spin filter. Be sure that before usage of Wash Buffer 2 you added 13 ml of 96% ethanol into the bottle with Wash Buffer 2. Centrifuge at 12,000 x g for 1 minute. Discard the flow through and discard the collection tube.

Background: Wash Buffer 2 is ethanol-based wash solution that cleans NA bounded onto the spin filter from residual impurities.

6. Put spin filter in new collection tube (provided). Centrifuge again at 12,000 x g for 1 minute to completely dry the spin filter membrane.
   Background: The spin filter is completely dried of ethanol residues to allow maximal NA release from the spin filter membrane in elution step.

7. Carefully remove the spin filter and transfer it into a new 2 ml Collection Tube (provided).

8. Add 50 µl of **Elution Buffer** onto spin filter and incubate 1 minute at room temperature.

9. Centrifuge at 12,000 x g for 1 minute.

10. Remove the spin filter unit. NA in the tube is now ready to use in any application. Please transfer isolated nucleic acids from Collection Tube to microtube with lid standardly used by your laboratory for nucleic acids storage.
    Background: Elution Buffer releases nucleic acids from the filter into the 2 ml Collection Tube. Nucleic acids are released due to no salt and no ethanol presence.

Performance characteristics

The kit was tested for the isolation of viral nucleic acids of influenza, viral hepatitis B, viral hepatitis C, coronavirus SARS-CoV-2 from clinical specimens such as buccal swabs, nasopharyngeal swabs, saliva and sputum. Subsequent testing using Real-Time PCR analyses and comparison with other commercial methods verified a high yield of viral NA from the samples.

The EliGene® Viral RNA / DNA FAST Isolation Kit for the isolation of viral nucleic acids from nasopharyngeal, oral, saliva and sputum swab specimens showed 100% agreement in SARS-CoV-2 RNA and influenza virus detection on 15 clinical specimens compared with a competitive isolation process. Furthermore, 100% reproducibility of the results was demonstrated. In 2000 clinical samples of nasopharyngeal swabs, inhibition of the subsequent PCR reaction was observed in only 0.2% of isolations.

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

Warnings and general precautions

This kit is intended for *in vitro* use only.

- **As SARS-CoV-2 is a serious pathogen, please follow actual WHO recommendations for BSL2+ or BSL3 laboratories!**
- **Lab safety gloves and respirators FFP3 are necessary for work with coronaviruses. Please work in appropriate biohazard boxes. Also centrifugation of samples must be performed in biohazard boxes. Keep in mind that the RNA of some viruses can also cause infection.**
- Handle and dispose of all biological samples as if they could transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying.
- Use all centrifuges, mini-centrifuges and vortexes in the Biohazard box only to prevent aerosol contamination.
- The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Dispose of all used tools, tips and work materials and clothing as potentially infectious and dispose of them in accordance with applicable regulations and recommendations for the handling of highly infectious waste.
- Keep in mind that all reagents and materials you work with may transmit infectious agents. Avoid direct contact with reagents. Waste must be disposed in accordance with adequate safety regulations. Consumables must be incinerated. Liquid wastes containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Wash hands carefully after handling samples and reagents.
- Work in standard mode of separate rooms: isolation, PCR set up, amplification, detection
- Dispose of leftover reagents and waste in compliance with adequate security measures.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer´s kit.
- Do not change recommended protocol!

Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

P280 Wear protective gloves/protective clothing/eye protection/face protection.
P281 Use personal protective equipment as required.

In case of any problems, please contact ELISABETH PHARMACON, Ltd.

Literature

Tansuphasiri U, Boonrat P, Rienthong S. Direct identification of Mycobacterium tuberculosis from sputum on Ziehl-Neelsen acid fast stained slides by use of silica-based filter combined with polymerase chain reaction assay. J Med Assoc Thai. 2004 Feb;87(2):180-9.

Liu X, Harada S. DNA isolation from mammalian samples. Curr Protoc Mol Biol. 2013 Apr;Chapter 2:Unit2.14. doi: 10.1002/0471142727.mb0214s102.

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5
615 00 Brno-Zidenice, Czech Republic
Phone: +420 542 213 851
E-mail: info@elisabeth.cz
Web: www.elisabeth.cz
VAT: CZ26258412

Symbols

	Catalog number
	Upper limit of temperature
	Batch code
	Use by (last day of month)
	*in vitro* diagnostic medical device
	Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests
	Attention, consult instructions for use
	Manufacturer

Manufacturer

ELISABETH PHARMACON Ltd.
Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827
E-mail: info@elisabeth.cz