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.

Table of Contents

Version: 11032020-02

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

Introduction

EliGene® Viral RNA/DNA FAST Isolation Kit is intended for **fast isolation (in 15 minutes)** of viral RNA and DNA (nucleic acids - NA) from serum, plasma, buccal swabs, sputum and saliva utilizing our special inhibitors removal technology. The isolation kit is intended for use with clinical samples containing low amount of nucleic acids. The isolated nucleic acid shows high purity suitable for successful RT-PCR amplification of RNA and DNA viruses present in the clinical specimens.

The principle of the isolation kit is based on lysis of viral particles by detergent. In the presence of chaotropic agent nucleic acids are bound onto spin filter, washed and eluted in TRIS-HCl buffer without EDTA. Nucleic acids are ready to use for RT-PCR, RT-qPCR and other downstream applications.

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

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*

Version: 11032020-02

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

NOTES BEFORE STARTING:

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. 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. Prepared Lysis Buffer is stable at room temperature for 24 hours. Keep prepared Lysis Buffer at room temperature!
Add 13 ml of 96% ethanol to Wash Buffer 2 before use!

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!

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. Add 200 µl of **serum, plasma,** Remel collection and transport **medium** to 1.5 ml Tube (not provided).
   If **dry buccal swabs** are processed, wash 1 – 3 swabs in 500 µl (or more if needed) of water for molecular biology or PBS buffer and take 200 µl of solution for nucleic acids isolation.
   Sputum or **saliva** should be dissolved in ratio 1:1 with PBS buffer and 200 µl of obtained solution should be used for nucleic acids isolation.

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. If EliGene® RT-qPCR detection kits from Elisabeth Pharmacon are used, add 10 µl of the Internal Control RNA provided with the kit in this step before vortexing.
   Background: Lysis Buffer is a lysis reagent containing detergent and other reagents required for complete cell lysis and binding of NA onto the silica membrane.

Version: 11032020-02

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 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.

Kit was tested for NA isolation of viruses (coronavirus, influenza virus, hepatitis B virus, hepatitis C virus) from clinical samples such as serum, plasma, buccal swabs, sputum and saliva. Subsequent RealTime PCR analysis confirmed high yields of viral nucleic acids in the samples. By comparison with other commercially available methods, identical or higher yields of nucleic acids were obtained.

Manufacturer:

ELISABETH PHARMACON, spol. s r. o.
Rokycanova 4437/5, Brno-Židenice 615 00, Czech Republic
info@elisabeth.cz | www.elisabeth.cz | tel.: +420 542 213 851

REF	Catalog number		Upper limit of temperature
LOT	Batch code		Manufacturer
	Use by (last day of month)	Σ	Contains sufficient "N" tests

Version: 11032020-02