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EliGene® Viral RNA/DNA FAST 96 Vacuum Isolation Kit
Instructions for Use

Package:

Ref. No.	Quantity
409480	480 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 96 Vacuum Isolation Kit is designed for rapid (less than 40 min) 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 silica 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.

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Required material not included in kit

480 × Microtube with lid used by customer for storage of isolated nucleic acids
65 ml of 96% ethanol for molecular biology for addition to Wash Buffer
72 ml of 96% ethanol for molecular biology for final washing

Required equipment

Microcentrifuge (15,000 × g)
Centrifuge for the 96 well plates (2,250 × g)
Incubator for 55°C
Vortex
Microtube rack
Pipettes: 1 – 1000 µl

Zephyrus Vacuum Pump Manifold System	ZVPMS-300
Zephyrus Vacuum Pump	ZVP-300
Zephyrus Vacuum Manifold UNI	ZVM-300
Zephyrus Vacuum Manifold 96	ZVM-96

Kit Contents

Components	Amount (480 isolations)
Lysis Buffer Stock Solution	102 ml *+ add Solution M*
Wash Buffer	190 ml *+ add 65 ml of 96% ethanol**
Elution Buffer	55 ml
Solution M	510 µl
96 Well Filter Plate	5 pcs
96 Deep Well Collection Plate	10 pcs
Cover foil on 96 Well Filter Plate	10 pcs

**65 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 1 µl of Solution M with 200 µl of Lysis Buffer Stock Solution. Add Solution M to the Lysis Buffer in a fume hood! In the case that you plan use all 480 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 65 ml of 96% ethanol to Wash Buffer before use! Use only ethanol intended for molecular biology, optionally 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 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 (Thermo 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.
Alternatively, it is possible to centrifuge 500 µl of the transport media at 15,000 × g for 5 minutes and collect 200 µl of supernatant from the surface for further analysis. This approach is recommended in the case of the clogging of the membrane due to the presence of saliva or sputum in the sample.
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 15,000 × g for 5 minutes. Then collect 200 µl of supernatant from the surface 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 96 Vacuum Isolation Kit** for the first time.

Important Notes before Using

Please wear gloves at all times.

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

Assemble the Vacuum Manifold according to the manufacturer's instructions. Waste tray has to be placed inside the base of the manifold. The 96 Well Filter Plate has to fit precisely on the top of the manifold. To allow the elution of the isolated nucleic acids, it has to be possible to assemble the 96 Deep Well Collection Plate into the base of the manifold.

In the case that less than 96 samples are isolated at once empty wells have to be sealed with the foil (supplied with the kit) to ensure appropriate vacuum conditions. Empty unused wells can be utilized in further isolation run. Empty (previously used wells) has to be sealed with the foil. It is recommended to finish the plate within 1 month from the first usage.

The format of the isolation allows utilization of the 8 channel pipettes or dispensers.

1. 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** into 96 Deep Well Collection Plate (supplied with the kit) The internal control according to the recommendations in the detection kit can be added in this step.

2. Pipette 200 µl of sample **(transport medium or diluted sputum or saliva)** to each deep well with 200 µl of Lysis Buffer from first step. Mix well by pipetting up and down for at least 5 times.

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. Transfer the whole liquid onto a 96 Well Filter Plate placed on the manifold, seal the plate with the Cover foil and apply vacuum until the solution has passed through the filter.
   Background: NA binds onto the silica membrane in the 96 Well Filter Plate 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** to the 96 Well Filter Plate. Be sure that before usage of Wash Buffer you added 65 ml of 96% ethanol into the bottle with Wash Buffer. Seal the plate with the Cover foil and apply vacuum until the solution has passed through the filter.
   Background: Wash Buffer is ethanol-based wash solution that cleans NA bounded onto the 96 Well Filter Plate from residual impurities.

5. Add 150 µl of **96% ethanol** on the 96 Well Filter Plate, seal the plate with the Cover foil and apply vacuum until the solution has passed through the filter.

6. Centrifuge the 96 Well Filter Plate at 2,250 × g at 1 min and then incubate the plate for 3 minutes at 55°C in the incubator. For the centrifugation, assemble the 96 Well Filter Plate into 96 Deep Well Collection Plate, which was used for the mixing of the sample with Lysis Buffer.
   Background: The 96 Well Filter Plate is completely dried of ethanol residues to allow maximal NA release from the silica membrane in elution step.

7. Assemble 96 Deep Well Collection Plate to the base of the manifold and place the 96 Well Filter Plate on the top of the manifold. Add 200 µl of the **Elution Buffer** directly on the filter and incubate for 3 minutes at room temperature. Seal the plate with the Cover foil and apply vacuum until the solution has passed through the filter.

8. Discard the 96 Well Filter Plate. NA eluted in the 96 Deep Well Collection Plate is now ready to use in any application. Please transfer isolated nucleic acids to microtubes with lid standardly used by your laboratory for nucleic acids storage.
   Background: Elution Buffer releases nucleic acids from the filter into the 96 Deep Well Collection Plate. Nucleic acids are released due to no salt and no ethanol presence.

Troubleshooting

Some transport media after mixing with the Lysis Buffer can form a foam during vacuum application. If this happens, pour 10 ml of ethanol to the waste tray to prevent foaming.

If the sample contains higher portion of mucous components (proteins and polysaccharides), the filter membrane can get clogged after transfer of the Lysis Buffer with the sample on the 96 Well Filter Plate. If this happens remove the liquid from the filter and repeat the NA isolation. When repeating isolation please consider the centrifugation of the sample at 15,000 × g for 5 min (see Primary sample collection, handling and storage Section for details).

To keep the vacuum at the desired level, the sealing of the whole plate after addition of the Lysis Buffer with the sample and Wash buffer with the included Cover foil is recommended. Sample with Lysis Buffer as well as Wash Buffer flow can differ in wells all over the 96 Well Filter Plate and sealing off the plate helps to keep the vacuum level on desired value. The Cover foil can be used repeatedly in steps 3, 4, 5 and 8 of the protocol.

If the sample is inhibited after the NA isolation (no signal of the internal control in the downstream qPCR reaction), it is likely that the residues of inhibitory compounds like ethanol are present in the sample. If this occurs, dilute the sample twice or increase the elution volume to 200 µl.

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.

Manufacturer

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