



EliGene[®] Urine Isolation Kit

REF 90051-50 (for 50 samples)

Kit components:

Proteinase K solution	1 ml
Solution MI3	12 ml
Solution MI4	12 ml
Solution MI5	27 ml
Solution MI6	27 ml
Solution MI7	12 ml
Spin Filters	50 pcs
2.0 ml Tubes	100 pcs
1.5 ml Tubes	50 pcs
Instruction for use	

Storage and shelf life after first opening:

All kit reagents and components of the kit must be transported at room temperature (15 - 30 °C).
After delivery Proteinase K solution must be stored at 2 - 8 °C.
When stored under these conditions, the kit will retain full activity until the expiration date indicated on the kit label.

Intended use

EliGene[®] Urine Isolation Kit is intended for DNA isolation from genomic and mitochondrial DNA from whole blood (fresh, frozen or stored at 4 °C), buffy coat, sperm, swabs, or cultured cells and from urine. This kit is optimized for usage with EliGene RealTime PCR kits.

Introduction

Many problems like inhibitions, low sensitivity etc. occurs when one make the isolation of DNA from clinical samples. EliGene[®] Urine Isolation Kit is designed to avoid all problems with PCR inhibitors and low yields of DNA. The total time of isolation process is about 1–2 hour including incubations. Kit contains all components necessary for the DNA isolation including microtubes. No addition of ethanol or other chemicals is needed.

Specimen

Clinical material: Whole blood (fresh, frozen or stored at 4 °C), buffy coat, swabs, sperm or cultured cells and urine.

Additional required equipment

- Automatic pipette 5–20 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Dry incubator or water bath for 65 °C.
- Centrifuge for microtubes (13000 x g).
- BIOhazard box.
- Lab safety gloves.



Precautions

Please wear gloves when using this product. It is recommended to work in BIOhazard box. Avoid all skin contact with reagents in this kit. In the case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (+420 542 213 851) or on our web site at www.elisabeth.cz. Reagents labeled flammable should be kept away from open flames and fire. Avoid contact with bleach or other oxidizers. This kit is intended for diagnostic use.

In the case of the usage of Proteinase K not supplied in this kit, use 20 µl of Proteinase K.

Procedure

1. a) **Urine:** Vortex whole volume of obtained urine for 15 seconds. Transfer 10 ml of vortexed urine to 15 ml falcon tube. Centrifuge urine at 6 000 x g for 20 minutes, discard supernatant and resuspend pellet in 200 µl of molecular grade water, **200 µl of MI3 solution**, and add **20 µl of Proteinase K** included in this kit and Internal Control from DNA diagnostic kit. Vortex for 15 seconds.

Incubate for 15 minutes at 65 °C.

Centrifuge lysate at 6 000 x g for 5 minutes. Transfer supernatant to microtube and discard sediment.

Add **210 µl of MI4 solution** to supernatant. Vortex for 15 seconds. Centrifuge briefly to collect the sample from the lid.

Transfer the lysate to the Spin Filter and centrifuge for 1 minute at 13,000 x g.

- b) **Blood:** Add **200 µl of whole blood or body fluids to a 1.5 ml Tube (provided)** and add **20 µl of Proteinase K** and Internal Control from DNA diagnostic kit.

Add **200 µl of MI3 solution** and mix by vortexing for 15 seconds.

Incubate the sample at 65 °C for 15 minutes. Centrifuge briefly to collect the lysate.

Add **210 µl of Solution MI4** and vortex for 15 seconds. Centrifuge briefly to collect the sample from the lid.

Transfer the lysate to the Spin Filter and centrifuge for 1 minute at 13,000 x g.

- c) **Swabs: Cervical swabs, urethral swabs, conjunctival swabs, swabs from rectum**

These specimens should be collected before the treatment of antibiotics according to standard protocol to collection tubes with transport medium for *Chlamydia* conservation (the kit was validated for transport medium remel MicroTest M4RT Transport and remel MicroTest M4 Transport). Specimens in these cultivation mediums should be transported to the laboratory at 4 °C. Specimens can be store at 4 °C up to seven days.

Recommended procedure for swabs specimens processing

Just before the DNA isolation vortex well the collection tubes for at least 10 seconds. Open the tube and by pressing the swab along the tubes wall press out the all solution from the swab. Discard the swab.

Centrifuge the collection tubes for 15 minutes at 6000 x g. If there is not centrifuge for collection tubes, split the medium in to the two 1.5 ml microtubes and centrifuge. Microtubes can be centrifuged 10 minutes at 10 000 x g.

Aspirate supernatant. From EliGene[®] Urine Isolation Kit add **200 µl of MI3 solution** and 200 µl of molecular grade water to the pellet and re-suspend the pellet by pipetting (if you used for the centrifugation two microtubes firstly re-suspend the pellet in one tube with given volumes of both buffer and consequently in other microtubes with the buffer mixture from the first one). Add **20 µl of Proteinase K** and Internal Control from DNA diagnostic kit.



Vortex for 15 seconds.

Incubate for 15 minutes at 65 °C.

Centrifuge lysate briefly to collect the sample from the lid and **add 210 µl of MI4 solution** to lysate.

Vortex for 15 seconds. Centrifuge briefly to collect the sample from the lid.

Transfer the lysate to the Spin Filter and centrifuge for 1 minute at 13,000 x g.

d) Sperm: For the DNA isolation from the sperm, use EliGene® Sperm Isolation Supplement (Cat.No. 90051-50-SPE) supplied by ELISABETH PHARMACON and follow instructions supplied in this supplement kit. EliGene® Sperm Isolation Supplement contains special buffers that are optimized for the isolation of DNA from sperm.

Other steps same for Blood, Urine, Sperm and Swabs:

2. Transfer Spin Filter to new 2 ml Tube (provided).
3. **Add 500 µl of MI5 solution** to spin filter. Centrifuge for 30 seconds at 13,000 x g.
4. Remove the spin filter and discard the flow through. Place spin filter back into the same 2ml collection tube.
5. **Add 500 µl of MI6 solution** to spin filter. Centrifuge 30 seconds at 13,000 x g.
6. Remove the spin filter and discard the flow through. Place spin filter back into the same 2ml collection tube.
7. Centrifuge again for 30 seconds at 13,000 x g to dry the spin filter membrane.
8. **Carefully remove spin filter** and transfer to a new 2 ml Tube (provided).
Add 100 µl of MI7 solution. To increase yields, incubate for 5 minutes at 65° C.
9. Centrifuge 1 minute at 13,000 x g.
10. Remove spin filter unit. Genomic DNA in tube is now ready to use for any application.

Interpretation of results

The whole process of DNA isolation must be followed by internal control. The internal control will provide the information about the susceptibility of DNA isolation and about possible PCR inhibitors. In the case of inhibited DNA sample it is best to use DNA samples prepared by new DNA isolation.

Alert

The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for DNA samples. Follow all recommendations for laboratories of DNA analyses.

Limitation of the examination procedure

The results obtained with this product are subject to the correct collection, transport, storage and preparation of samples. To avoid result mistakes it is therefore necessary to take particular care during these phases and to carefully follow provided instructions.

This product must be handled by personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid result errors.

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.



This product requires the use of special clothing and instruments for extraction/preparation of amplification reactions and for amplification/detection of amplification products to avoid false positive results.

As with any diagnostic device, the results obtained with this product must be interpreted in consideration of all the clinical data and other laboratory tests done on the patient.

Warnings and general precautions

This kit is intended for in vitro use only.

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. 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.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste 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.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- 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 kits.

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.



- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- 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

- These solutions carry the following safety warnings (P; H):

Solution MI3

H302+H332	Harmful if swallowed or if inhaled.
H315	Causes serious skin irritation.
H319	Causes serious eye irritation.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312+P330	If swallowed: Call a poison center or doctor/physician if you feel unwell. Rinse mouth.
P304+P340+P312	If inhaled: Remove person to fresh air and keep comfortable for breathing. Call a poison center or doctor/physician if you feel unwell.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/ attention.

Solution MI4

H225	Highly flammable liquid and vapor.
H319	Causes serious eye irritation.
H336	May cause drowsiness or dizziness.
P210	Protect from heat/sparks/open flame/hot surfaces. No smoking.
P233	Keep the container tightly closed.
P305+P351+P338	In case of eye contact: Rinse cautiously with water for several minutes. Remove contact lenses, if possible and if easy to remove. Continue rinsing.



Solution MI5

H225	Highly flammable liquid and vapor.
H315	Causes serious skin irritation.
H319	Causes serious eye irritation.
P210	Keep away from heat/sparks/open flames/hot surfaces. No smoking.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

Solution MI6

H225	Highly flammable liquid and vapor.
H315	Causes serious skin irritation.
H319	Causes serious eye irritation.
P210	Keep away from heat/sparks/open flames/hot surfaces. No smoking.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

Solution MI7

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

Proteinase K solution

H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P304+P340	If inhaled: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P342+P311	If experiencing respiratory symptoms: Call a poison centre or doctor/physician.
P284	Wear respiratory protection.



Literature

Barbara A. Bannister, Norman T. Begg and Stephen H. Gillespie: Infectious Disease. Blackwell Science, 2th Ed., 2000

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, spol. s r.o.

Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827

E-mail: info@elisabeth.cz