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EliGene® MTB Isolation Kit

REF 90043-50 (for 50 samples)

Kit components:

Lysozyme	5 x 1,4 x 10^6 units
Proteinase K solution	1 ml
Solution MI1	100 ml
Solution MI2	6 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 and Lysozyme 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® MTB Isolation Kit is intended for DNA isolation from *Mycobacterium species* and eventually from further microorganisms (G+) from clinical materials as sputum, decontaminated sputum, cultures, BAL, exudates, urine, swabs.

This kit is optimized for usage with MTB Q-PCR Alert kit and EliGene kits.

Introduction

Many problems like inhibitions, low sensitivity etc. occurs when one make the isolation of DNA from *Mycobacterium* species. EliGene® MTB Isolation Kit is designed to avoid all problems with PCR inhibitors and low yields of DNA. The total time of isolation process is about 2 hours including two incubations, each for 30 minutes. Kit contains all components necessary for the DNA isolation including microtubes. No addition of ethanol or other chemicals is needed.

Specimen

Clinical material: Sputum, decontaminated sputum, cultures, BAL, exudates, urine, swabs

Additional required equipment

- Automatic pipette 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 37-95 °C.
- Centrifuge for microtubes (13000 x g).
- BIOhazard box.
- Lab safety gloves.

Precautions

Please wear gloves when using this product. In the case of MTB DNA isolation, it is necessary to work in BIOhazard box. Avoid all skin contact with reagents in this kit. In 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.

Reagent preparation

Before the start of DNA isolation prepare Lysis buffer: add 1 ml of MI2 solution to the tube with Lysozyme and vortex briefly. This solution is stable for 14 days at 4 °C.

Procedure

1. a) Sputum, BAL, exudates and bacteria from culture

   Resuspend sample in the same volume of MI1 solution and centrifuge at 14 000 x g for 10 minutes. In the case of high viscosity of sample, add MI1 solution.

   Aspirate supernatant and resuspend pellet in 100 µl of Lysis buffer (MI2 solution with lysozyme).

   Incubate for 30 minutes at 37 °C.

   b) Urine, decontaminated sputum and BAL

   Centrifuge urine, decontaminated sputum or BAL at 14 000 x g for 10 minutes and resuspend pellet in 100 µl of Lysis buffer (MI2 solution with lysozyme).

   Incubate for 30 minutes at 37 °C.

   c) Swabs

   Put swab into microtube with at least 2ml of MI1 solution and vortex on high speed for 2 minutes. Discard swab and centrifuge at 14 000 x g for 10 minutes. Aspirate supernatant and resuspend pellet in 100 µl of Lysis buffer (MI2 solution with lysozyme).

   Incubate for 30 minutes at 37 °C.
2. **Add 100 µl of MI3 buffer together with 20 µl of Proteinase K**. 20 µl of Internal Control to lysate should be added in the case that you will use isolated DNA for another analysis by MTB Q-PCR Alert kit. Vortex and incubate for 30 minutes at 65 °C.
3. At the end of incubation increase the temperature to 95 °C and incubate mixture for another 10 minutes at 95 °C. Thereafter vortex the sample at full speed for 1 minutes and spins it shortly for removing drops from cap. Let stand the sample for 5 minutes to decrease the temperature of lysate to room temperature.
4. **Add 100 µl of Solution MI4** and vortex for 15 seconds. Briefly centrifuge to remove drops from the top of the lid. Transfer all of the mixture to the spin filter. Centrifuge 1 minute at 13,000 x g.
5. 5. Transfer spin filter to new 2.0 ml tube. **Add 500 µl of Solution MI5** to spin filter. Centrifuge 30 seconds at 13,000 x g. Remove spin filter, discard flow through, and replace spin filter.
6. **Add 500 µl of Solution MI6** to spin filter. Centrifuge 30 seconds at 13,000 x g. Remove spin filter, discard flow through, and replace spin filter. Centrifuge again for 30 seconds at 13,000 x g. Remove spin filter carefully and transfer to a new 2.0 ml tube without it coming in contact with the wash.
7. **Add 50 µl of Solution MI7** preheated in aliquots on 65 °C. Incubate for 5 min. Centrifuge 1 minute at 13,000 x g. Remove filter unit and close tube lid. Genomic DNA in the 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.

Performance characteristics

Diagnostic performance characteristics:

Within the frame of testing the functional characteristics of EliGene® MTB Isolation Kit kit overall 559 clinical specimens were analyzed. From these specimens 518 blind specimens from Prague region and 41 MTB positive specimens verified by cultivation form Brno region were analysed. From these 559 clinical samples 52 samples were MTB positive by other laboratory methods (microscope, cultivation, MGIT). The kit EliGene® MTB Isolation kit succesfully isolated MTB DNA from all clinical MTB positive specimens. 26 specimens were inhibited during the PCR – these samples were identified by other methods as MTB negative.

Sensitivity of the Isolation kit showed 100 % in isolation of DNA from clinical decontaminated material which means that this procedure for isolation of MTB DNA gives in 100% sufficient amount of MTB DNA for the next DNA diagnostic method. The effectiveness of these isolation procedures is 95,34% which means that from total amount of isolated specimens were isolated 95,34 % of specimens without inhibition.

A negative result obtained with this product suggests that the MTB DNA was not detected in DNA extracted from the sample, but it may also contain MTB DNA at a lower titre than the detection limit for the product; in this case the result would be a false negative.

Internal control of quality

The internal control must be used during MTB DNA isolation to avoid false negative results caused by PCR inhibitors.

Limitation of the examination procedure

- This kit is intended only for following human clinical samples: sputum, decontaminated sputum, BAL, exudate, urine. Bacterial cultures can be also used.
- This kit is not intended for the isolation of MTB DNA from the blood.
- The results obtained with this product are subject to the correct collection, transport, storage and preparation of samples. To avoid result errors 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.
- As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product. This residual risk cannot be eliminated or reduced any further. In particular situations such as emergency diagnoses, this residual risk can contribute to incorrect decisions with potentially grave consequences for 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.
- In the case of any problems call our customer support.

Warnings and precautions specific to components of the kit

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

Solution MI1

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

Solution MI2

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

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.

Lysozyme

H335	May cause respiratory irritation.
P260	Do not breathe dust/fumes/gas/mist/vapours/spray.

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

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