DNA diagnostics EliGene®

CE

EliGene® MTB RT

REF **90030-RT (for 50 samples)**

Intended use:
EliGene® MTB RT kit is qualitative in vitro diagnostic device intended for the detection of bacteria *Mycobacterium tuberculosis* in clinical material or bacterial cultures.
EliGene® MTB RT kit has a very high sensitivity that allows detect even 10 genomic DNA in the mastermix.
Specificity of the kit: kit provides positive result for *M. tuberculosis*, *M. bovis*, and negative result for *M. kansasii, M. xenopi, M. avium* and *M. marinum.*

Introduction:
Genus *Mycobacterium* comprises more than 50 species when among them obligatory pathogenic etiological agents of tuberculosis and leprosy and on the other hand also facultative pathogenic and non-pathogenic species representing important part of naturally biotopes can be found. *M. tuberculosis* comprises to the one complex with genetically close related *M. bovis* and it is etiological agents of human tuberculosis. *M. bovis* is etiological agents of cattle tuberculosis and some others pets and wild beast and also rarely human.

Kit components:
5 x 200 µl **MTB Mix**
1 x 125 µl Positive Control DNA – **PC DNA MTB**
5 x 200 µl Internal Control DNA – **IC DNA MTB**
Instruction for use

Specimen:
For diagnostic purposes following clinical specimen are recommended a) sputum, b) BAL, c) exudate, d) urine, e) other clinical material.

Clinical material:	Recommended DNA isolation procedure:
Sputum, BAL, exudate, urine	EliGene® MTB Isolation Kit (Cat. No 90043-50)

For MTB Isolation we recommend to use EliGene® MTB Isolation Kit that is CE certified as IVD for MTB DNA isolations. This kit is optimized for usage with EliGene® MTB RT kit. EliGene® MTB Isolation Kit is suitable for MTB DNA isolation from sputum (BAL, urine, exudate) and/or decontaminated MTB samples appointed for MTB cultivation. The efficacy of this isolation kit is 95,34%, the sensitivity of isolation by this kit is 100%.

Essential equipment in laboratory:
Sterile 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

Equipment for Real-Time PCR – the kit is designed for Real-Time Systems 7000, 7300, 7500 from Applied Biosystems and is fully compatible with RotorGene instruments without other processing.
Sterile micro tubes (DNase-, RNase- free) compatible with given Real-Time system.

Storage and expiration date: all components of the kit must be transported and stored at -20° C. Once the reagents are thawed do not freeze them again and store them at 4°C. Under these conditions they are stable at least for 14 days. If after the analysis remaining part of the reagents aliquots do not freeze them and store them in fridge until the next analysis. Under these conditions they are fully functional at least for 14 days. Kit must be store in a dark.

Configuration of Real Time instrument:
Use the program module for absolute quantification (Plate Type "Absolute Quantification")

					95° C 15 s
1x	50° C 2 min	1x	95° C 10 min	45x	60° C 1min

For MTB detection probe labeled with Yakima Yellow is used (similar to VIC – abs. 525nm – emission 548 nm, similar to JOE abs. 520nm – emission 548nm).
For internal control probe labeled with FAM is used (abs. 494nm – emission 518nm).
Collect emission signal at 60°C.
Mastermixes include passive reference control dye ROX for signal normalization.
Mastermixes include amplificates contamination protection.

Procedure:

1. During the isolation 20 µl of Internal Control DNA (IC DNA MTB) must be added to the sample. Take the isolation of DNA by optimized protocol of EliGene® MTB Isolation Kit.
2. Take one micro tube of MTB mix and after the thawing pipette 20 µl of mix to amplification tube and add 5 µl of isolated DNA. Tube with MTB mix do not freeze again and idle content store at 4° C in a dark.
3. Positive control: Take off one micro tube MTB mix and after the thawing pipette 20 µl of mix to amplification tube and add 5 µl of Positive control from Positive control DNA (PC DNA MTB) tube.
4. Insert the micro tubes to the Real-Time PCR instrument and run the program.

Results viewing:
Positive result is characterized by increasing of amplification signal in Yakima Yellow channel (eventually VIC). When the result is negative or slightly positive the amplification of internal control must be observed in FAM channel. In the case of strongly positive samples usually the internal control amplification is not detected.

For the RealTime System 7000, 7300 and 7500 use the automatic analysis. The values of Ct correspond to the quantity of positive result, "result undet." means negative result. Positive result is characterized by increasing of fluorescence signal in specific channel.

Results interpretation:

Negative result
If the increasing of amplification signal in Yakima Yellow channel (eventually VIC) does not appear before cycle number 45, the result of test should be interpreted as probably MTB DNA negative. The signal for internal control must be positive – see article Quality control. This result does not exclude the occurrence of MTB infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed MTB DNA.

Positive result
MTB DNA was detected in the sample. The sample is MTB DNA positive.

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

Inhibited sample
In the case that increasing of amplification signal in Yakima Yellow channel and also increasing of amplification signal in FAM channel is not observed, it is necessary to repeat the analysis. Best is to use DNA samples prepared by new DNA isolation.

Quality control:
EliGene® MTB RT Kit involves internal isolation control and positive control. Internal isolation control follows the quality of DNA isolation and detects mistakes in the isolation process. It detects the occurrence of an inhibition of amplification process. In the case that the sample is MTB DNA negative, the Ct of internal control must be Ct < 30.

Positive control
Positive control follows the proper function of mastermix. Minimal Ct of positive control must be 27 or lesser. The Ct higher than 27 for positive control can't be accepted and DNA detection must be repeated with new sample. In the case of repeatedly higher Ct contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use the water for molecular biology used in your laboratory. For negative control use the pipette for DNA samples.

Troubleshooting:

1. No amplification of internal control can be caused by a) strongly positive result of MTB amplification b) in the case of negative result for MTB there is some problem in isolation of DNA or the kit is after the expiration date or there is Real-Time instrument breakdown.
2. If there is no amplification of positive control, the kit is after the expiration date or there is Real-Time instrument breakdown.

Principle of the method:
This diagnostic kit uses primers and TaqMan probes (labeled by FAM and YakimaYellow).

Functional characteristics:

Kit EliGene® MTB RT has a very high sensitivity - detects 10 genomic DNA added to the amplification mix.

The sensitivity of test was verified as follows.

Pure culture of *M. tuberculosis* was re-suspended in physiological solution and bacterial number was calculated microscopically. Dilutions were prepared when 0, 1, 10, 50, 100, 200 bacteria were added to mastermix. Totally it was tested for three times. Detection of *M. tuberculosis* was successfully for all samples containing 10 and more bacteria. The addition of human DNA in usual concentration has no effect to the sensitivity of the method.

Specificity of the methods was validated by sequence comparison of used probes and primers with DNA databases (GenBank, http://www.ncbi.nlm.nih.gov/) and by addition of human DNA to mastermix. 50 different samples of human DNA gave no false positive result. Moreover, addition of DNA from *M. kansasii, M. xenopi, M. avium* a *M. marinum,* HBV, EBV, CMV, HSV1, HSV2, VZV, *C. trachomatis, E. coli, A. niger, C. albicans* and isolates from vague spirochetes from arthropods did not give false positive result too.

Repeatability of the method was tested with 10 positives and 10 negatives samples for three consecutive days. The same lot of mastermix was used. The results were same in all performed tests.

The sensitivity of reaction depends on handling with specimen (isolation of DNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Limitations of the method:

With this product use only DNA extracted from the following human samples: sputum, decontaminated sputum, BAL, exudate, urine.

Do not use DNA extracted from heparinized samples with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

Do not use DNA extract that is contaminated with haemoglobin with this product: haemoglobin inhibits the amplification reaction of nucleic acids and may causes invalid results.

There are no data available concerning inhibition caused by antiviral drugs, chemotherapeutic drugs or immunosuppressants.

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 the instructions provided with the products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real-time amplification assay of nucleic acids used in this product is subject to contamination from MTB-positive clinical samples, positive controls and the amplification reaction products themselves. Contamination leads to false positive results. The product has been designed in such a way as to reduce contamination; nevertheless, this phenomenon can only be prevented by following good laboratory practices and by complying scrupulously with the instructions provided in this manual.

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.

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 (see paragraph on Performance Characteristics); in this case the result would be a false negative.

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.

Clinical trials:
Clinical trials were completed in two Czech clinical laboratories. Totally 559 clinical samples were tested by EliGene® MTB RT Kit, 518 of them were blind samples with unknown status from Prague region (including samples of foreigners) and 41 MTB positive samples verified by cultivation from Brno region. From 559 samples, 52 were MTB positive. EliGene® MTB RT Kit detected correctly all 52 positive samples. 26 samples were inhibited during PCR method but these samples were MTB negative by other methods. Totally 481 samples were correctly diagnosed by EliGene® MTB RT Kit as MTB negative.

In clinical trials were found the specificity and sensitivity 100% for EliGene® MTB RT Kit.

The calculation of sensitivity and effectiveness of MTB DNA isolation carried out by **EliGene MTB Isolation Kit:**
100% sensitivity on 559 clinical decontaminated samples (sputum, BAL, urine, exudate) was found. It means that using this method of MTB DNA isolation in 100% cases was isolated sufficient quantity of MTB DNA that was receivable by DNA diagnostics. The effectiveness of this MTB DNA isolation is 95,34%. It means that from all isolated clinical samples were 95,34% samples without an inhibition.

Warning:
After thawing, Mastermixes are stable for 2 weeks at 4°C. Do not freeze Mastermix again! Do not mix components of the kits from different lots!

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 manufacturers' 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

The tubes containing mixes (MTB Mix, Inhibition Control Mix – IC Mix MTB) are disposable and therefore must be used once only in the preparation of the reaction mixture.

These mixes carry the following safety warnings (S):

S 23-25. Do not breathe gas/fumes/vapor/spray. Avoid contact with eyes.

The tubes containing mixes (Inhibition Control DNA – IC DNA MTB, Positive Control DNA – PC DNA MTB) are disposable and therefore must be used once only in the preparation of the reaction mixture.

These mixes carry the following safety warnings (S):

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In the case of any problems call our customer support.

References:

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