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BIOTUB Kit

Kit for Mycobacterium tuberculosis DNA detection in human clinical samples

Instructions for Use

Ref. 90.073

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

BIOTUB Kit

For research use only (RUO)
Not for use in diagnostic procedures
Test results may be used for preliminary analysis only

Some of the applications which may be performed with this product may be covered by applicable patents in certain countries. The purchase of this product does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on the country and/or application. Biotools does not encourage the unlicensed use of patented applications.

PLEASE CHECK INTEGRITY OF KIT AND REAGENTS BEFORE USE. DETERIORATED KITS MAY CAUSE EQUIVOCAL RESULTS.

INTENDED USE

The BIOTUB Kit is a method for the determination, in a qualitative form, of *Mycobacterium tuberculosis* in clinical samples. The detection is achieved in two steps. The first step consists of an amplification detecting sequences specific for *Mycobacterium tuberculosis*. The second step is a nested amplification reaction, which can be performed at user's will, in order to achieve the maximum sensitivity of the test.

The BIOTUB Kit is to be used with the following samples:

- Blood samples stored in EDTA. Use of blood samples with heparine or citric acid has not been tested, and therefore, the kit may not render the expected results.
- Lung tissues. For the use of the Kit with biopsies do not treat the tissue with acetic acid or iodide. Paraffin-embedded tissues can be used provided the employed fixative reagents do not degrade or interact with DNA and deparaffination step is performed.
- Sputums. Yield of the reaction will depend on the amount and quality of DNA present in the sputum, and therefore, poor-quality sputum samples may render equivocal results.

SUMMARY AND EXPLANATION OF THE TEST

Tuberculosis (TB), in most cases caused by *Mycobacterium tuberculosis*, is one of the main cause of death by infectious agents worldwide. Number of cases has increased dramatically in the last decades, with around 8 million new cases and 3 million deaths reported each year[1]. Though TB incidence has started to show a decrease in cases in the last few years in developed countries, appearance of resistance strains has posed a serious problem for health management.

M. tuberculosis expands through the air and infects the lung and other tissues. Though the mere presence of *M. tuberculosis* in an individual is not always associated with development of TB or even ability to spread it to other individuals, an early indication is a must for an efficient patient management. It has been observed that immunodepression may lead to the development of acute TB disease for individuals that harboured the pathogen for months, even years, without presenting any symptom.

Traditional techniques for the detection of *Mycobacterium tuberculosis* include bacterial culture in differential media, which may elapse up to 4-8 weeks until growth is observed[2]. In contrast to detection of *Mycobacterium tuberculosis* by culture methods, DNA amplification techniques may shorten clinical detection from weeks to less than a day[3]. However, and associated with the increase in TB cases, resistance in *M. tuberculosis* strains has proved to be an important problem, as it leads to genetic differences that may affect the quality of the diagnosis by molecular methods[4], specially as far as rifampin resistance is concerned[5].

The BIOTUB Kit is an amplification test for the qualitative detection of *Mycobacterium tuberculosis* DNA, and has been developed for use with a variety of clinical samples.

[1] Yuen *et al.* (1999). J Clin Microbiol, 37 (12): 3844-3850.
[2] Fukushima *et al.* (2003). J Clin Microbiol, 41 (6): 2605-2615.
[3] Down *et al.* (1996). J Clin Microbiol, 34 (4): 860-865.
[4] Quan *et al.* (1999). Antimicrob Agents Chemother, 43 (1): 181-184.
[5] Heep *et al.* (2001). J Clin Microbiol, 39 (1): 107-110.

PRINCIPLES OF THE PROCEDURE

BIOTUB Kit is a test for qualitative analysis, using a DNA amplification technique for direct detection of DNA through agarose electrophoresis and ethidium bromide staining. Bacterial DNA present in positive samples is specifically amplified by using specific primers, which hybridise with homologous sequences in the bacterial genome.

The kit consists of two amplification reactions, using two pair of primers. The firs reaction uses one pair of primers (Pair 1 – MT1 and MT2) hybridising with conserved sequences specific to *Mycobacterium tuberculosis* and non homologous to other *Mycobacterium* species (*IS6110* region), and therefore, indicates *Mycobacterium tuberculosis* presence. Use of conserved regions is indicated in order to avoid genetic variabilities associated with antibiotic resistance. The amplified fragment is highly conserved between different *M. tuberculosis* isolates known so far. Primer Pair 1 has a high Tm, and therefore, annealing and elongation steps are performed simultaneously at 72 ºC, which increases the specificity of the reaction. Use of high-Tm primers also allows the performance of high cycle number-amplification rounds (55-70 cycles).

The second reaction is a nested amplification, using as template the product from the first amplification reaction. By using a second pair of primers (Pair 2 – MT3 and MT4), sensitivity of the detection is increased several-folds.

The first amplification renders a band of approximately 219 bp, while the second amplification renders a product of approximately 123 bp. For band analysis, agarose electrophoresis must be performed, followed by ethidium bromide staining.

The detection process using BIOTUB Kit consists of three main steps: sample preparation, bacterial DNA amplification and detection by agarose electrophoresis and ethidium bromide staining.

Sample Preparation

The BIOTUB Kit is used with DNA purified from clinical samples.

NOTE

For bacterial DNA purification, use of the SPEEDTOOLS DNA EXTRACTION KIT (Ref. 21.130, 21.131, 21.132) or SPEEDTOOLS TISSUE DNA EXTRACTION KIT (Ref. 21.135, 21.136, 21.137) is recommended. Use of other methods is also possible. However, the user must confirm that the purified DNA can be used with the BIOTUB Kit (concentration 50-100 ng/µl, $A_{260/280}$=1.8 – 2.0, absence of inhibitors that may affect the amplification, etc.). It is recommended to check the quality and suitability of the purified DNA for amplification reactions, for example, by performing control amplifications in parallel. For further information, please contact our Technical Dpt. (info@biotools.eu).

Amplification and Detection

Target selection

Selection of the target *Mycobacterium tuberculosis* sequences has been based on the study of highly conserved regions in the *M. tuberculosis* genome (MT1 and MT2 primers). The selected bacterial genome region (*IS6110*) has a high degree of conservation between the tested *Mycobacterium tuberculosis* isolates. Pair 1 (MT1 and MT2) defines a segment of approximately 219 bp, while Pair 2 (MT3 and MT4) defines a sequence of approximately 123 bp in *Mycobacterium tuberculosis*.

Amplification

DNA amplification is performed with a thermostable DNA polymerase from *Thermus*. In the presence of magnesium, and with the suitable salt and ionic strenght conditions, the enzyme shows DNA polymerisation activity using as anchor a primer and a DNA molecule as template.

DNA purified from the sample to be analysed is added to the reaction mix in the corresponding amplification vial. The amplification vial contains all necessary reagents to perform the amplification reaction, and only DNA and sterile bidistilled water must be added. Reaction mixture, already containing DNA, is incubated at different temperatures, in order to allow the hybridisation of the primer pairs to the DNA from the sample. Once hybridisation of primer pairs has taken place, and in the presence of triphosphate deoxynucleotides, the DNA polymerase extends the primer and forms a DNA strand complementary to the template DNA. Cyclic repetition of this process results in exponential amplification of the sequences originally present in the sample included between the primer pairs.

Detection

Detection of amplified products is performed by agarose gel electrophoresis followed by ethidium bromide staining.

NOTE

Ethidium bromide is a highly mutagenic intercalating agent. We recommend use of gloves and the maximum caution in handling.

Mycobacterium tuberculosis presence is indicated by a band of approximately 219 bp. If the second amplification is performed in order to increase the sensitivity of the test, a 123 bp band will appear.

REAGENTS

The Kit contains amplification reagents in liquid format for performance of 96 amplification reactions (Ref. 90.073). Thaw and handle reagents on ice. Do not freeze/thaw Kit vials repeatedly. In case of frequent use, we recommend the aliquoting of the vial contents.

- **Amplification Mixture:** Three vials: 3 x 1470 µl
  A Tris-HCl solution, containing <10 % glycerol, KCl, <0.001 % dATP, dCTP, dGTP, dTTP and primers. TB amplification mixture includes all amplification reagents for the detection of *M. tuberculosis*, except $MgCl_2$ and DNA polymerase, in the adequate ratios.
  Store at -15±8ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly. For frequent use, we recommend the aliquoting of the vial contents.

- **Nested Mixture:** Three vials: 3 x 1470 µl
  A Tris-HCl solution, containing <10 % glycerol, KCl, <0.001 % dATP, dCTP, dGTP, dTTP and primers. TB nested mixture includes all amplification reagents for the detection of *M. tuberculosis*, except $MgCl_2$ and Biotools DNA polymerase, in the adequate ratios.
  Store at -15±8ºC. Thaw and handle on ice. Do not freeze/thaw repeatedly. For frequent use, we recommend the aliquoting of the vial contents.

- **50 mM $MgCl_2$ Solution:** Vial: 1.8 ml
  Store at -15±8ºC. Thaw on ice. Mix well before use.

- **Biotools DNA polymerase (1 U/µl):** Vial: 210 µl
  Store at -15±8ºC. Add to reaction mixtures shortly before introduction of vials in thermal cycler

- **Positive Control:** Vial: 300 µl
  Non-infective positive control consisting of DNA amplified product (10^5 copies/µl), containing a generic sequence from *Mycobacterium tuberculosis*, flanked by primers. This positive control can be used as intra-tube control.
  Store at -15±8ºC. Thaw and handle on ice. Avoid repeated freeze/thaw cycles. For frequent use, aliquot the content of the vial.

MATERIALS REQUIRED BUT NOT PROVIDED

NOTE

For all equipments, regular maintenance and calibration is necessary. Follow manufacturer's instructions, and check working parameters regularly, specially for thermal cyclers and pipettes. Maintenance and calibration of instruments allows its correct functioning, and helps detecting problems that may render an incorrect analysis result.

Pre-amplification area

- Equipment, reagents and disposable material necessary for DNA purification (depending on the method, follow manufacturer's instructions)
- Timer

- Automatic pipettes[6] (10, 20 and 200 µl), filter or positive displacement tips, RNase-free[7]
- Disposable examination gloves, powder-free
- Sterile bidistilled water
- Screw cap polypropylene tubes, 1.5 ml capacity, non siliconised, conical, sterile, RNase-free. It is recommended to use screw cap tubes, in order to avoid the potential contamination of samples and controls.
- Racks for 1.5 ml vials
- Containers for disposal of potentially-infectious material
- Disposable filter paper for working surface, cleaning paper for accidental spills
- Termi-DNA-Tor[8] or equivalent, in order to remove DNA from working surfaces

Amplification area

- Thermal cycler: Eppendorf MasterCycler™ Personal, MJ Research MiniCycler™ or Applied Biosystems GeneAmp™ 2700. Use of this kit in other equipments has not been tested. For further information, contact our Technical Dpt. (info@biotools.eu)
- Laminar flow cabinet
- Racks for reaction vials
- Reaction vials (0.2 ml, thin-walled)
- Sterile bidistilled water (Ref. 20.033 or equivalent)
- Automatic pipettes (10, 20 and 200 µl), filter or positive displacement tips, RNase-free
- Disposable examination gloves, powder-free
- Containers for disposal of potentially-infectious material
- Disposable filter paper for working surface, cleaning paper for accidental spills
- Termi-DNA-Tor or equivalent, in order to remove DNA from working surfaces

Post-amplification area

- Electrophoresis power supplies and tanks
- Gel Documentation system
- UV transilluminator
- Ethidium bromide
- Low EEO agarose (Ref. 20.011) or equivalent
- TAE or TBE
- DNA Ladder ranging between 150 to 700 bp (Ref. 31.006) or equivalent
- Electrophoresis loading buffer
- Automatic pipettes (10, 20 and 200 µl), filter or positive displacement tips, RNase-free
- Disposable examination gloves, powder-free
- Protective mask / goggles for UV
- Microwave

WARNINGS AND PRECAUTIONS

Following is a list of warning and precautions. For more information, we recommend to read the Material Safety Data Sheet (MSDS), available in our webpage (www.biotools.eu), or by request to our Technical Dpt. (info@biotools.eu).

A. Research Use Only.
B. This test must be used with clinical samples collected, handled and stored as indicated in the corresponding chapter. Efficiency of the test in other samples has not been tested.
C. The kit detects *Mycobacterium tuberculosis,* and does not detect other *Mycobacterium* species (specially, no cross-reaction with *Mycobacterium avium* has been tested). Though the kit is based on the use of highly conserved regions (*IS6110*), where no mutations associated to resistance have been described to our knowledge, new mutants may appear affecting these region, and therefore, performance of the test.
D. Handle all samples and discarded material as infectious or potentially infectious.
E. Use powder-free examination gloves while handling reagents or samples, as well as lab coat. Wash hands thoroughly after performing the test.
F. Open and close reagent vials carefully. Observe temperature and light exposure instructions. After use, close vials and store at indicated temperatures.
G. All materials used with the kit, including reagents and samples, must be discarded as to inactivate all possible infectious agents
    1. **Solids**: autoclave

[6] Precision of automatic pipettes must be in the range of 3 % of the indicated volume. If necessary, calibrate and check regularly, following manufacturer's instructions. It is recommended to use RNase-free filter tips and positive displacement tips, in order to avoid cross contamination between samples and amplicons.
[7] It is recommended to use different sets of pipettes for each reaction step (pre-amplification, amplification, post-amplification), in order to avoid contaminations that may render false positive results.
[8] Available in Biotools' catalogue (Ref. 40.201).

2. **Liquids**: add sodium hypochloride[9] at a final concentration of 1 %, and incubate 30 minutes at room temperature before discarding any material.

H. Spills: wash spills with a 5 % solution of sodium hypochloride. Cover surface with absorbent material, saturated with a 5 % solution of sodium hypochloride. Let at least for 10 minutes. In order to avoid fume exposure, a plastic or glass cover can be used. All materials used for washing spills must be treated as infectious or potentially infectious material.
I. Do not use product after expiry or best before date.
J. Kit components have been tested as a whole. **Do not interchange components** with other kits, or components from different lots.
K. Nucleic acids are very sensitive to degradation by nucleases. Nucleases are present in human skin and surfaces that have been in contact with humans. Wash with Termi-DNA-Tor and cover working surfaces with suitable paper. Use powder-free examination gloves throughout the whole process.
L. Extreme care must be taken when aliquoting the different volumes in each reaction step. Mix well after addition of each reagent, unless otherwise noted. Read instructions for use of automatic pipettes.
M. Do not pipette by mouth.
N. Packaging material included with the kit is resistant to the indicated storage conditions. Storage at different conditions can cause breakage of the material, and possible contamination of kit contents.
O. Plastic material included with the kit is resistant in the normal conditions of use. Use of plastic material in extreme conditions may cause its breakage, and therefore, impossibility to use the kit.
P. Kit reagents, once used, must be discarded. Reagents cannot be reused once they have been used for the analysis of clinical samples, as this may cause false positive or false negative results.
Q. Laboratory workflow must be unidirectional, from pre-amplification area to amplification area. Specific equipment for each working area must be used, in order to avoid cross contaminations. Equipment used for amplification must remain in this area at all times.

STORAGE AND HANDLING INSTRUCTIONS

1. After reception, store the different reagents at the indicated temperatures at -15±8ºC. Use non frost-free freezers. Also, for frequent use (more than 1 time a week), aliquot the contents of the vials in different tubes, in order to avoid repeated freeze/thaw cycles.

2. Do not use the kit after expiry date. The closed kit is stable until the indicated date, if storage instructions are correctly followed. Do not mix reagents from other kits and/or other lots. If trace amounts of reagents remain, they must be discarded.

SAMPLE COLLECTION, TRANSPORT AND STORAGE

The different samples recommended for use with the BIOTUB Kit are indicated at the beginning of this Manual. Samples taken with other methods or transported according to different specifications have not been tested for use with the kit. Tissue samples must be collected before applying acetic acid or iodide.

Samples stored in EDTA

Samples may be stored up to three days at room temperature (maximum 25 ºC), and shipped without refrigeration to the clinical analysis laboratory. For prolonged storages, we recommend temperatures of 2-8 ºC, or freezing. Once in the laboratory, they can be stored at 2-8 ºC if analysis is to be performed in one week. If the analysis is to be performed later, store samples at -15±8ºC. Samples must be kept away from heat sources and preserved of environmental humidity. Bacterial growth must be avoided and DNA integrity must be kept.

Sputum samples

Samples should be stored at temperatures of 2-8 ºC, or freezing; and sent by express courier (maximum 24 hours) to the laboratory. Once in the laboratory, they can be stored at 2-8 ºC if analysis is to be performed in one week. If the analysis is to be performed later, store samples at -15±8ºC. Samples must be kept away from heat sources and preserved of environmental humidity. Bacterial growth must be avoided and DNA integrity must be kept.

Lung biopsies

Fresh biopsies of up to 5 mm must be used. Biopsy must be immediately placed in a preservative medium and stored at -15±8ºC. Biopsies can be shipped by express courier (24 hours maximum) at a maximum

[9] Commercial bleach usually contains sodium hypochloride at a concentration of 5 %. Bleach can be used, after performing the necessary calculations in order to achieve the indicated concentration.

temperature of 25 ºC, and stored at -15±8ºC in the laboratory until performance of the test. Biopsies with a diametre under 2 mm must not be used.

Use of paraffin-embedded lung tissues is possible provided that tissue fixation method do not degrade DNA and purification of DNA is performed with methods specific for this kind of sample. For informative purposes, a protocol for purification of DNA from paraffin-embedded tissues is provided in our webpage (www.biotools.eu/eng/productos/paraffin.pdf).

In order to avoid accidents or accidental opening of the sample container, it is recommended to seal its closure with Parafilm® or equivalent before freezing.

Sample shipment must comply with local, national and international regulations for transport of etiological agents.

INSTRUCTIONS FOR USE

NOTE

Thaw all reagents on ice. Keep on ice while in use.

Amplification must be started in the next 10 minutes after adding the purified DNA and controls to the amplification mix.

Check thermal cycler regularly. Non-existent or poor calibration of the equipment may render equivocal results.

1.- Final reaction volume is 50 µl. Calculate the necessary volume of **Amplification Mixture, $MgCl_2$**, **Biotools DNA Polymerase** and **Positive Control** for the analysis of samples and controls. It is recommended to perform one Positive Control and one Negative Control in each round of analysis (this must be taken into account when calculating necessary volume for performance of all reactions).

2.- Mix the necessary volume of **Amplification Mixture, $MgCl_2$** and **Biotools DNA Polymerase** for the number of reactions to perform in a 1.5 ml vial. **Perform this process in a laminar flow cabinet**. Keep the reaction mixture (reaction mixture = Amplification Mixture + $MgCl_2$ + Biotools DNA Polymerase) on ice:

Reagent	For 3 reactions	For 10 reactions	For n reactions
Amplification Mixture	126 µl	420 µl	42 * n µl
50 mM $MgCl_2$ Solution	6 µl	20 µl	2 * n µl
Biotools DNA Polymerase (1U/µl)	3 µl	10 µl	1 * n µl

3.- Aliquot 45 µl of the reaction mixture in each amplification vial, **in the laminar flow cabinet.**

4.- Remove vials from laminar flow cabinet. Add 50-100 ng from DNA from the purified samples and/or controls to each amplification vial. Complete up to 50 µl final volume with sterile bidestilled water.

NOTE

For Positive controls use 5 µl of Positive Control vial. For negative controls use 5 µl of sterile bidistilled water.

Clinical samples, depending on their nature and infection levels, may have different DNA concentrations, and therefore, amount of template to be added to the amplification reaction is expressed in ng rather than indicating sample volume. Quantity and purity of template must be calculated, e.g. by measuring $A_{260/280}$ values.

5.- Close amplification vials. Place them in thermal cycler. Store remaining of all reagents at -15±8ºC.

Perform the amplification according to the following program:

94 ºC	3 min	
94 ºC	30 sec	55-60 cycles
72 ºC	30 sec	
72 ºC	7 min	
4 ºC	∞	

NOTE

This protocol has been adapted for Eppendorf, MJ Research and Applied Biosystems GeneAmp™ thermal cyclers. For other thermal cyclers, optimisation of reaction parameters may be necessary. For any question, please contact our Technical Dpt. (info@biotools.eu).

6.- Store amplified reactions at 2-8 ºC. Store at room temperature only if electrophoresis or amplification is performed within 1-2 hours.

Nested Amplification

For samples negative after the first amplification, a second amplification round may be performed in order to increase the sensitivity of the test.

1.- Final reaction volume is 50 µl. Calculate the necessary volume of **Nested Mixture, $MgCl_2$**, **Biotools DNA Polymerase** and **Positive Control** for the analysis of samples and controls. It is recommended to perform one Positive Control and one Negative Control in each round of analysis (this must be taken into account when calculating necessary volume for performance of all reactions).

2.- Mix the necessary volume of **Nested Mixture, $MgCl_2$** and **Biotools DNA Polymerase** for the number of reactions to perform in a 1.5 ml vial. **Perform this process in a laminar flow cabinet**. Keep the reaction mixture (reaction mixture = Nested Mixture + $MgCl_2$ + Biotools DNA Polymerase) on ice:

Reagent	For 3 reactions	For 10 reactions	For n reactions
Nested Mixture	126 µl	420 µl	42 * n µl
50 mM $MgCl_2$ Solution	6 µl	20 µl	2 * n µl
Biotools DNA Polymerase (1U/µl)	3 µl	10 µl	1 * n µl

3.- Aliquot 45 µl of the reaction mixture in each amplification vial, **in the laminar flow cabinet.**

4.- Remove vials from laminar flow cabinet. Add 5 µl from the product from the first amplification and/or controls to each amplification vial. Complete up to 50 µl final volume.

NOTE

For Positive controls, use 5 µl of Positive Control vial. For negative controls, use 5 µl of sterile bidistilled water.

5.- Close amplification vials. Place them in thermal cycler. Store remaining of all reagents at -15±8ºC.

Perform the amplification according to the following program:

94 ºC	3 min	
94 ºC	30 sec	40 cycles
63 ºC	30 sec	
72 ºC	30 sec	
72 ºC	7 min	
4 ºC	∞	

NOTE

This protocol has been adapted for Eppendorf, MJ Research and Applied Biosystems GeneAmp™ thermal cyclers. For other thermal cyclers, optimisation of reaction parameters may be necessary. For any question, please contact our Technical Dpt. (info@biotools.eu).

6.- Store amplified reactions at 2-8 ºC. Store at room temperature only if electrophoresis is performed within 1-2 hours.

INTERPRETATION OF RESULTS

The analysis of amplification products is performed by horizontal electrophoresis in low EEO-agarose gels (e.g. MB Agarose, Ref. 20.011). Band visualisation is improved in 1.5-2 % gels using TAE 1X or TBE 0.5X as running buffers. It is recommended to add ethidium bromide in the agarose gel for a better resolution and visualisation.

NOTE

Ethidium bromide is a highly mutagenic intercalating agent. Use of gloves and maximum caution is recommended on handling this reagent.

Samples containing *Mycobacterium tuberculosis* DNA should render a 219 bp band (or a 123 bp band for samples that have been submitted to the Nested Reaction).

QUALITY CONTROL

It is recommended that at least one (1) Positive Control and one (1) Negative Control be run each time the test is performed. As with any new laboratory procedure, novel users should consider performing additional controls (both positive and negative) until a high degree of confidence is reached.

The Positive Control must render a 219 bp band (or a 123 bp band if Nested Reaction has been performed). Vials containing negative control (sterile bidistilled water) must render no bands. Any analysis not fulfilling any of these results must be completely invalidated and discarded. It is necessary to repeat the process from its beginning, including DNA purification, processing other aliquot of the original sample. A failure of instruments during the test, indicated by error messages, also means that the test has not been valid. Repeat all the procedure for each sample from the amplification step.

For laboratories requiring an external control, this must contain a defined number of copies of the target sequence for *Mycobacterium tuberculosis*, and the bacteria levels in this control must be a multiply of the limit value of the testing procedure.

PROCEDURAL PRECAUTIONS

1. Laboratory workflow must be unidirectional, from pre-amplification area to post-amplification area. Pre-amplification tasks must be initiated with the preparation of the reagents and sample purification. Equipments, materials and reagents must be dedicated and they must not be used for other activities or be transferred from one to another area. Gloves must be worn in each area, and must be discarded before proceeding to the next area. Equipments and materials used for setting-up of reactions must not be used for other activities, or for pipetting or processing amplified DNA or other DNA sources.

2. As with any analytical procedure, it is fundamental to use a good laboratory practice to obtain good results with this technique. Due to the high analytical sensitivity of the test, extreme care must be taken in order to keep the purity of all kit reagents and all reaction mixes. All reagents must be carefully checked in order to ascertain their purity. Discard all suspect reagents.

PROCEDURAL LIMITATIONS

1. Research Use Only.

2. Instructions must be followed in order to obtain correct results. Should the user have any questions, please contact our Technical Dpt. (info@biotools.eu).

3. This test has been validated for use with the reagents provided by the kit. The use of other amplification methods, or the use of equipment not fulfilling the specifications, may render equivocal results. User is responsible for validating the modifications for this test, in any of the indicated parameters.

4. This test has been validated with samples collected and shipped as per the "Sample Collection" chapter. Any modification has not been validated, and therefore, obtained results may not be correct.

5. Detection of *Mycobacterium tuberculosis* DNA depends on the number of bacterial particles present in the sample, and can be affected by sample collection methods, patient-related factors (e.g. age, symptoms), or for infection stage and sample size.

6. False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives.

7. Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.

8. Use of this product is limited to qualified professional personnel, experienced in DNA purification and DNA amplification techniques.

9. It is important to pipette the indicated amounts, and mix well after each reagent addition. Check pipettes regularly.

WARRANTY

Products are guaranteed to conform to the quality and content indicated on each vial and external labels during their shelf life. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.

Any complaint on damaged goods during transport must be directed to the handling or transport agent.

Product for Research Use Only. This product must be used by qualified professionals only. It is the responsibility of the user to ascertain that a given product is adequate for a given application. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including, without limitation, implicit warranties of commercialisation ability or adequacy for a given purpose, are provided by BIOTOOLS. BIOTOOLS will not be held responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product.

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2008 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
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