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# Trueprep® AUTO

# MTB Sample Pre-treatment Pack

**Sample pre-treatment pack for MTB samples**

## 1. INTENDED USE

**Trueprep® AUTO** MTB Sample Pre-treatment Pack **(REF 60204AS05 / 60204AS20 / 60204AS25 / 60204AS50 / 60204AS100 / 60204AS200)** is used to liquefy and pretreat the pulmonary and EPTB specimen before proceeding for extraction and purification of nucleic acids using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device.

## 2. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused predominantly by the bacillus *Mycobacterium tuberculosis.* It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB). Over 80% of TB infections are pulmonary and the diagnosis is largely based on analysis of sputum sample. However, when sputum production is not possible, Bronchoalveolar Lavage (BAL) is used as a non-sputum sample. The remaining about 20% of TB infections is extra pulmonary (EPTB) and occurs in various body fluids and tissues. There is no good reference diagnostic standard for EPTB. Culture, histopathology and Nucleic Acid Amplification Tests (NAAT) such as PCR are the most commonly used direct diagnostic methods. Culture and histopathology requires specialized and controlled laboratory facility and highly skilled manpower and takes days to weeks to provide result. PCR, especially Real Time PCR is sensitive, specific and provides results in a few hours. The **Truelab®** Real Time Quantitative micro PCR System and **Truenat® MTB/ Truenat® MTB Plus** makes real time PCR technology rapid, simple, robust and user friendly and offers "sample to result" capability in about one hour even at resource limited settings. The PCR process necessitates the extraction and purification of nucleic acids from clinical specimens, to free it from potential PCR inhibitors. The **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device together with **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit provides an easy method of nucleic acid extraction and purification for further PCR on **Truenat® MTB / Truenat® MTB Plus.** The **Trueprep® AUTO** MTB Sample Pre-treatment Pack allows for processing of pulmonary and EPTB specimen for further extraction by **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit.

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**The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007 (Patent No. 281573), WO 2009/047804 and corresponding claims of any foreign counterpart(s) thereof.**

**The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.**

**The Truenat® MTB Chip-based Real Time PCR test is protected by the following patents and patents pending: IN 796/CHE/2012 and corresponding claims of any foreign counterpart(s) thereof.**

## 3. PRINCIPLE OF THE TEST

**Truenat® MTB / Truenat® MTB Plus** requires purified nucleic acids from pulmonary and EPTB specimen. Non-sputum samples are paucibacillary in nature characterized by very low loads of MTB bacilli and samples such as tissue, pus, abscess etc. have non-homogeneous distribution of the bacteria. Also, these samples tend to have high levels of PCR inhibitors. Hence it is necessary to digest such complex specimen to release the bacteria, concentrate to get better yields of Bacilli and also discard potentially inhibitory substances. The **Trueprep® AUTO** MTB Sample Pre-treatment Pack employs a combination of reagents to achieve these objectives and to enable further extraction and purification of the bacterial DNA using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device together with **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The pre-treated sample is added to the sample chamber of the cartridge of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The cartridge is then placed in the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device for processing. DNA from the sample are bound by the matrix and inhibitors present in sample are washed out. At the end of processing the bound DNA is eluted and collected in elution chamber. All the waste generated in the process is contained within the dump region of cartridge. The elute is transferred to the Elute Collection tube (ECT).

## 4. DEVICE DESCRIPTION AND PRODUCT SPECIFICATION

**Trueprep® AUTO** MTB Sample Pre-treatment Pack (REF 60204AS05 / 60204AS20 / 60204AS25 / 60204AS50 / 60204AS100 / 60204AS200) is an add-on pack containing reagents to liquify and pre-treat pulmonary and EPTB samples before proceeding for extraction and purification of nucleic acids using the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. Proceed with instructions mentioned in packinsert of **Truenat® MTB/MTB Plus** for further Real Time PCR analysis of processed sample for the quantitative detection and diagnosis of *Mycobacterium tuberculosis* (MTB).

## 5. CONTENTS OF Trueprep® AUTO MTB Sample Pre-treatment Pack

One kit of **Trueprep® AUTO** MTB Sample Pre-treatment Pack consists of the following components as per the Pack size 5T / 20T / 25T / 50T / 100T / 200T:

A. Liquefaction buffer
B. Lysis buffer
C. Disposable transfer pipette (graduated) - 1ml
D. Package Insert

| REF | 60204AS05 | 60204AS20 | 60204AS25 | 60204AS50 | 60204AS100 | 60204AS200 |
|---|---|---|---|---|---|---|
| Σ | 5T | 20T | 25T | 50T | 100T | 200T |

## 6. STORAGE AND STABILITY

**Trueprep® AUTO MTB** Sample Pre-treatment Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

## 7. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

1. Disposable micro pestle (for tissue homogenization)
2. Microcentrifuge and tubes (2 ml)
3. Centrifuge and tubes (for upto 10 ml volume)
4. Sterile water
5. **Truelab®** Real Time micro PCR Workstation (REF 623010001 / 633010001 / 643010001 / 653010001) consisting of
    1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001/603042001).
    2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF603021001 / 603022001 / 603023001).
    3. **Truelab®** micro PCR Printer (REF 603050001).
    4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
    5. **Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat®** Positive Control Panel - I, (REF 801010008), Powder free disposable gloves and waste disposal container with lid.

## 8. TYPE OF SPECIMENS, VOLUME NEEDED FOR TEST AND STORAGE

| Sr. No. | Sample Type | Sample volume |
|---|---|---|
| 1. | Bronchoalveolar Lavage (BAL) | 5-10ml |
| 2. | Pleural fluid, Peritonial fluid | 5-10ml |
| 3. | Cerebrospinal fluid | 0.5 ml |
| 4. | Pus,Abscess | 0.5 ml |
| 5. | Lymphnode aspirate | 0.5 ml |
| 6. | Tissue/ Biopsy samples | 100 mg approx. |
| 7. | Sputum | 0.5 ml |

Fresh specimen must either be processed immediately as per sample procedure outlined in section on Sample processing protocol or stored frozen at -20°C. Frozen samples must be brought to room temperature before starting sample processing.

**Note:** Frozen specimen should not be subjected to more than 3 freeze/thaw cycles as this can lead to erroneous results.

## 9. SAMPLE PROCESSING PROCEDURE

**Truenat® MTB** requires purified nucleic acids from pulmonary and EPTB specimen that are extracted using the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. Samples must be liquefied and pre-treated using the **Trueprep® AUTO** MTB Sample Pre-treatment Pack provided, as per protocol below, before proceeding for extraction.

**For sputum samples:**

Check if the specimen is pipettable. If not, add 1 drop of liquefaction buffer to the specimen (If specimen is frozen allow it to reach room temperature first). Allow the reagent to hydrate the sample by swirling gently. Incubate at room temperature for 5 minutes. If sample has not liquefied after 5 minutes, incubate for another 5 minutes until sample is pipettable. This depends on sample viscosity and ambient temperature.

Label a lysis buffer tube with patient ID and transfer 500 µl of the liquefied sample into the lysis buffer tube using the graduated disposable transfer pipette provided. Add 2 drops of the liquefaction buffer to the lysis buffer tube and mix gently. Close

the cap tightly and mix well. Wait for 3 minutes.
Check if contents are fully liquefied by shaking the tube. If not, incubate it further till the contents are liquefied. Depending upon the sample, this may take another 10 to 15 minutes. Do not proceed if the content has not liquefied.

**For non- sputum samples:**

A. Protocol for BAL, Pleural fluid, Peritoneal Fluid
1. Take appropriate volume (Refer section 8) of the sample in a tube.
2. Spin at 4000x g for 5 minutes.
3. Discard the supernatant until 500µl remains at the bottom and then add 2 drops of Liquefaction buffer to the sample.
4. Transfer all the entire contents to Lysis Buffer tube from **Trueprep® AUTO MTB** Sample Pre-treatment Pack and leave it for 5 minutes.

B. Protocol for Pus, Abscess, lymph node aspirate and CSF
1. Take appropriate volume (Refer section 8) of the sample in a tube.
2. Add 2 drops of Liquefaction buffer to the sample.
3. Transfer all the contents to Lysis Buffer tube from **Trueprep® AUTO MTB** Sample Pre-treatment Pack and leave it for 5 minutes.

C. Protocol for Tissue / Biopsy Samples
1. Tissue samples must first be homogenized by using 100µl Lysis Buffer using micro pestle.
2. Collect homogenized sample and add 2 drops of Liquefaction buffer.
3. Transfer liquefied sample to Lysis buffer tube from **Trueprep® AUTO MTB** Sample Pre-treatment Pack and leave it for 5 minutes.

**Note:** If un-dissolved tissue remains, transfer only the clear fluid to Cartridge provided in the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit.

**Sample Storage and Transportation:**
Sample Pre-treatment decontaminates the specimen and makes it ready for extraction. Sample in this form is stable for 3 days at upto 40°C and 1 week at 30°C .

**Nucleic acid extraction:** Follow Extraction procedure (Section-13) of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit package insert (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit for details**).** ⚠ Dispose off the lysis buffer tube, and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 14).

## 10. SAFETY PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20 - 30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the User Manuals of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and package inserts and Material Safety Data Sheets (MSDS) of **Truenat® MTB / Truenat® MTB Plus** and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit.
5. All materials of human origin should be handled as though potentially infectious.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

## 11. PROCEDURAL PRECAUTIONS
1. Do not exchange kit components from different lots.
2. Check all packaging before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However if the outer packaging is damaged the user must check that components of the kit are intact before using them.
3. Do not perform the assay in the presence of reactive vapours (e.g. from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
4. All pipetting steps should be performed with utmost care and accuracy. Cross-contamination between reagents and samples may invalidate results.

## 12. PROCEDURAL LIMITATIONS
1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truenat®** assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or specimens.
4. A specimen for which the **Truenat®** assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truenat®** assay should be interpreted in the context of other clinical and laboratory findings.

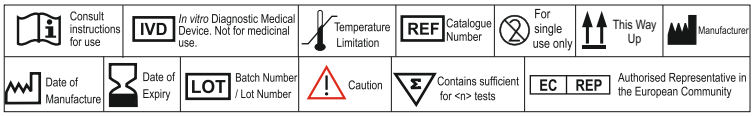
## 13. CLEANING AND DECONTAMINATION
1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared sodium hypochlorite [10 times dilution of 5% sodium hypochlorite (household bleach)] before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves, should be disposed off as potentially bio-hazardous waste e.g. in a biohazard waste container.

## 14. DISPOSAL AND DESTRUCTION
1. Submerge the used content such as transfer pipette, pipette tips, lysis buffer tube, EPTB microcentrifuge tubes, Disposable micro pestle etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% sodium hypochlorite for 10 volumes of or water).
4. Do not autoclave materials or solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

## 15. REFERENCES

1. Robert L., Janet T., Elizabeth S., et al. (2022) Practical Guide to Specimen Handling in Surgical Pathology. College of American Pathologists.
2. Kayvan Z., Vaigundan D., Kutty M., et al. (2019) An efficient and cost-effective method for purification of small sized DNAs and RNAs from human urine. Plos One. 1-15.
3. Castelo A., Jardim J.R.B., Goihman S. (1989) Comparison of daily and twice weekly regimens to treat pulmonary tuberculosis. Lancet. 2: 1173-76.
4. Siun C.T & Beow C.Y. (2009) DNA, RNA, and Protein Extraction: The Past and The Present. Journal of Biomedicine and Biotechnology. 1-10.
5. Pratt K.D., Sylvain G., Arnaud B., et al. (2018) Optimized Lysis-Extraction Method Combined With IS6110-Amplification for Detection of Mycobacterium tuberculosis in Paucibacillary Sputum Specimens. Frontiers in Microbiology. 9: 1-10.
6. Cleaning/Disinfection SOP for Research Laboratories for Mitigating DNA Contamination. 2021. Boston University.


**SYMBOL KEYS**





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