Instructions for Use

Actiphage® TB PCR Test

Intended for the detection of mycobacteria belonging to *M. tuberculosis* complex (MTBC) in human blood samples.

For research use only

100 Reactions

Catalogue No. PBD-PC-009

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1 INTENDED USE

PBD Biotech's Actiphage® TB PCR Test is a molecular assay developed for the specific detection of mycobacteria, belonging to the *Mycobacterium tuberculosis* Complex (MTBC), in blood and this procedure describes this application for human blood samples.

Purification and concentration of the mycobacterial genomic DNA may be required, prior to the PCR assay, depending on the sample type and tissue of source. We recommend using the Monarch® PCR & DNA Cleanup Kit (New England BioLabs) or the DNA Clean-up and Concentrator (Zymo Research) following the manufacturer's instructions.

2 PRINCIPLE OF ACTIPHAGE® TB PCR TEST

The Actiphage® TB PCR Test contains a lyophilized PCR bead allowing homogeneous detection of:

- Mycobacteria belonging to MTBC by targeting the insertion sequence IS6110 with hydrolysis probe FAM labelling, with a limit of detection of one genomic copy.
- An Exogenous internal amplification control IAC DNA, with hydrolysis probe Cy5 labelling, to confirm absence of PCR inhibitors.

This PCR test has been validated on a Cobas® z 480 Analyser 96-well thermal block cycler unit (Roche Diagnostics) with halogen lamps using FAM (465-510) and Cy5 (610-670) detection channels. Software version: LCS480 1.5.1.62 SP3 - UDF 2.1.0.26.

The PCR lyophilized bead contains Uracil N-glycosylase (UNG) to eliminate potential post-PCR carryover contamination associated with routine molecular testing.

3 PRODUCT CONTENTS

Each test contains sufficient materials for 100 reactions (4 x 25 reactions). The shelf life of each component is indicated on the respective label.

The test must be stored at 2 °C – 8 °C on receipt but is shipped between 10 °C and 24 °C (ambient temperature).

3.1 ITEMS PROVIDED

1 x Actiphage® TB PCR Test (PBD-PC-009) containing:

- 4 x TB Lyophilized PCR Beads

- 4 x PBD Resuspension Buffer x2
- 4 x Negative Control (NTC - water)
- 4 x Internal Amplification Control (IAC)

3.2 ITEMS REQUIRED BUT NOT PROVIDED

3.2.1 Reagents

- Genomic DNA from MTBC to use as a PCR Positive Template Control (PTC) (Optional).

3.2.2 Accessories and Equipment

General Equipment:

- 'Pre-PCR Mix' and 'DNA preparation' dedicated laboratory areas
- Fridge (2 °C – 8 °C)
- Freezer (-20 °C – -18° C)
- Pipettes capable of dispensing 1 mL, 200 µl, 100 µL, 20 µL, 10 µL and 1 µL.
- Microcentrifuge suitable for 2 mL microcentrifuge tubes and capable of generating at least 16,000 x g.
- Centrifuge capable of spinning 96 well plates at a maximum 500 x g.
- Vortex mixer
- Quantitative PCR (qPCR) Instrument
- Howie style side fastening laboratory coat.
- Safety glasses

General Disposables:

- Containers for discarding of waste (various sizes).
- Disposable gloves
- Sterile disposable 1.5 mL microcentrifuge tubes.
- Sterile filtered pipette tips for all listed pipette volumes.
- qPCR plates (96 well) and adhesive seals

3.3 STORAGE, RECONSTITUTION, SHELF-LIFE, AND RE-USE OF TEST COMPONENTS

Prior to use, the test components should be stored at 2 °C – 8 °C. Ensure the components to be used are removed from the fridge and brought to room temperature (RT; 18 °C – 25 °C) prior to starting the assay.

The components should not be used beyond the expiry date shown on the Actiphage TB PCR Test label.

To ensure optimal performance, it is important that all test components are reconstituted as directed below and that any unused test components are stored according to the following instructions.

Reconstitute the TB lyophilized PCR bead (red cap) by adding 280 µL of PBD Resuspension Buffer x2 (yellow cap).

One TB lyophilized PCR bead vial is sufficient for 25 reactions.

Resuspended PCR reagents are stable at 2 °C – 8 °C for three weeks, but no longer than the expiry date.

4 ACTIPHAGE® TB PCR TEST PROCEDURE

1. Establish qPCR plate setup defining each sample position including Actiphage® positive and negative controls and NTC.
2. In a 'Pre-PCR Mix' dedicated area, prepare a Master Mix by mixing 10 µL of resuspended PCR bead solution (prepared as per section 3.3) and 5 µL of IAC (green cap) per reaction.
3. Transfer 15 µL of Master Mix into each well of interest.
4. In the 'DNA Preparation' dedicated area, add 5 µL of each DNA sample in the corresponding well.
5. Add 5 µL of nuclease-free water (blue cap) in the NTC well.
6. Seal the plate and spin down before placing it in the thermocycler.
7. Run the following qPCR program settings.

Table 2. qPCR program settings.

Cycles	Time	Temperature
UNG*	2 minutes	25 °C
1 cycle start	5 minutes	95 °C
40 cycles	10 seconds	95 °C
	1 minute	60 °C

*Activation step for Uracil N-glycosylate. Alternatively, once PCR samples and reagents are combined in the plate allow to stand for at least 2 minutes at RT to allow activation of UNG.

NOTE: An optional PCR Positive Template Control (PTC) can be included by adding 15 µL of Master Mix and 5 µL of quantitative genomic DNA from MTBC. An optional PCR Negative Control (NC) can be included by mixing 15 µL of resuspended PCR bead and 5 µL of NTC (water).

5 ACCEPTANCE CRITERIA

5.1 PCR Controls

The threshold cycle (Ct or Cp) is the intersection between the amplification curve and the threshold line. It allows the relative measurement of the concentration of the target in the PCR reaction when a reference sample is analysed in the same run. To analyse and interpret the signals obtained by qPCR, the threshold must be set up. On the Cobas z 480 Analyser the FAM and Cy5 channels are individually analysed using “AbsQuant/Fit points” method using the default settings (Background 2-6, Auto baseline and Auto threshold calling).

The qPCR results are validated if the controls (NTC, IAC, NC, PTC) present valid results (see table 3).

To validate the assay, the PCR negative control sample (NTC and NC) must not exhibit a qPCR signal in the relevant target channel (FAM).

IAC is a reaction-independent control designed to co-amplify a genomic region other than our target, using the same reagents (except probe), under the same annealing temperature and reaction conditions. It controls for machine or PCR reagent failure.

To validate the assay from an IAC perspective, any samples with IAC (Cy5) Ct values ≥ 35 should be considered invalid and repeated.

Table 3. Acceptance criteria for PCR controls.

Control	Ct (FAM)	Ct (Cy5)	Interpretation
NTC - Negative Template control	≥ 40 <40		Plate Pass Plate Fail
IAC - Internal Amplification Control		< 35 ≥ 35	Sample Pass Sample Fail
NC - Negative Control (Optional)	≥ 40 <40		Plate Pass Plate Fail
PTC - Positive Amplification Control (Optional)	≥ 40 <40		Plate Fail Plate Pass

5.2 Test samples

Once the PCR and Actiphage controls are validated as described in section 5.1 and 5.2, sample results can be interpretated according to the table below.

Table 5. Interpretation of test samples results.

Targets		Interpretation
MTBC (FAM)	**IAC (Cy5)**	
Negative	Positive	Negative
Positive	Positive	Positive
Positive	Negative	Invalid - Repeat analysis Presence of inhibitors, competition with the main target or omission of IAC addition.
Negative	Negative	Invalid - Repeat analysis DNA omission, degradation or not in contact with Master Mix. Presence of inhibitors or omission of IAC addition.

Negative: FAM Ct ≥ 40 or Cy5 Ct ≥ 35.
Positive: FAM Ct < 40 or Cy5 Ct < 35.

6 LIMITATIONS

Detection of mycobacteria by the Actiphage® TB PCR Test is dependent on the quantity and quality of the sample collected, its storage prior to processing, and the number of live organisms present.

A false positive result will only occur if there is cross-contamination of the sample with mycobacterial DNA from the target organism.

7 PRECAUTIONS

7.1 TECHNICAL PRECAUTIONS

- If the Actiphage® TB PCR Test appear damaged upon arrival, do not use and contact PBD Biotech.
- Actiphage® TB PCR Test reagents must not be used beyond the expiry date printed on the label.
- Do not substitute the reagents in the test or mix between batches or lot numbers, as this may affect performance.
- Do not freeze any test component or reconstituted reagent.
- The reagents are provided at defined working concentrations. Assay performance may be compromised if reagents are modified or not used or stored under recommended conditions, as detailed in section 3.3.
- Reconstitute sufficient reagents required to perform the test. Actiphage PCR Test contains reagents for a total of 100 reactions that can be run in batch sizes of 25.
- All reagents must be brought to RT before use.
- Use aseptic and PCR contamination control techniques, as appropriate, throughout the reagent preparation and assay procedure. All pipettes, pipette tips and plastic-ware used must be sterile and or DNA/ RNAse free.
- Care must be taken to treat all samples and control reactions in the same manner. Omission of, or deviation from, any step could lead to inaccurate results.
- 'Pre-PCR Mix' and 'DNA preparation' need to be carried out in two physically separated areas to reduce the chance of contamination.
- Use individual, dedicated pipets for setting up reaction mixes and adding positive control reagents.
- Use extreme caution to prevent contamination of the controls and reaction mix reagents with the synthetic materials that are contained in the IAC reagent.
- Do not use qPCR reaction volumes (reaction mix plus sample) of less than 20 µL.
- Do not open the PCR plate after the qPCR run has finished.

- Refer to the Cobas® z 480 Analyser user manual (available at www.diagnostics.roche.com) for additional qPCR instrument warnings, precautions, and procedures.
- Caution must be observed to ensure correct sample testing with emphasis on wrong sample entry, loading error, and pipetting.
- To prevent cross-contamination between samples leading to false positive results, it is mandatory to use disposable materials for single use and to avoid direct contact between samples.
- Any instrumentation, or connecting parts, including (but not limited to) qPCR instruments, centrifuges and pipettes must be calibrated, maintained, and cleaned as directed by the manufacturer.
- The operator should seek assistance directly from PBD Biotech if they do not understand anything in these instructions or have problems running the test.

8 MATERIAL SAFETY DATA SHEETS

A material safety data sheet (MSDS) for this test is available from PBD Biotech, upon request.