Instructions for Use (IFU)

Product Name : EMPE TBDx

Qualitative Real-Time PCR test for the detection of *Mycobacterium tuberculosis* complex.

Catalogue Number : TBDx-25

Pack Size : 25 reactions

For Research use only (RUO)
NOT for diagnostic or clinical use

RUO

1. General Information

1.1. Contents

Component	Volume (µl)
Master mix	1 vial of 737.5
Probe mix	1 vial of 12.5
ROX (Optional)	1 vial of 7.5

1.2. Storage and Stability

- When stored at −15 to −25°C, the kit is stable through the expiry date printed on the label. Maximum of Six freeze thaw cycles are allowed.

1.3. Additional Equipment and Reagent required

- **Consumables:** PCR tubes/plates, adhesive films, filter tips, micropipettes, gloves, nuclease-freewater.
- **Equipment:** Real-time PCR instrument, extraction system, centrifuge, vortex mixer, freezer, refrigerator.

1.4. Intended use

- The EMPE TBDx assay is a qualitative real-time PCR test intended for the detection of *Mycobacterium tuberculosis* complex (MTBC) DNA from extracted nucleic acid in bacterial cultures as well as human respiratory specimens such as sputum or bronchoalveolar lavage (BAL). The assay is for research use only and not intended for use in diagnostic procedures or patient management.

1.5. Principle of the Test

- The assay is based on real-time PCR technology using MTBC-specific primers and probes. Amplification and detection occur simultaneously, with fluorescence monitored in real time. An internal control monitors validity of assay setup and PCR inhibition.

2. How to Use This Product

2.1. Before You Begin

- Specimens: sputum and BAL. Store at 2 – 8°C up to 24 hours or -20°C for longer. Follow safety precautions and good laboratory practices.

2.2. Sample Preparation

- The EMPE TBDx assay utilizes an in-house DNA extraction protocol. Briefly, 1 ml of sputum or BAL sample is decontaminated, followed by heat lysis of the sediment using sample preparation buffer. 10µl of the crude lysate is used directly as template DNA for the assay.

Note: Customer can also validate their own extraction process using other DNA extraction Kits.

2.3. PCR setup protocol

- Thaw required reagents completely. Vortex gently and centrifuge briefly all vials before setting up the PCR run.

Component	Volume per reaction (µl)
Master mix	**29.5**
Probe mix	**0.5**
Extracted DNA	**10**

NOTE: Keep the reagents at (5 ± 3) °C for the shortest time possible until the PCR reaction is set up.

- The total reaction mix volume is 40 µl. Close the tubes, vortex gently, centrifuge shortly, insert them into the real-time PCR device and amplify according to the following PCR profile.

2.4. PCR Profile

Temperature °C	Time	Stage	Signal collection
37	5 min	Hold	No
95	5 min	Hold	
95	10 sec	40 cycles	
64	30 sec		
72	30 sec		YES*

- *Plate read/Data acquisition in FAM, VIC/JOE/SUN, in Bio-RadTM CFX 96 and Rotor gene.Q For Applied biosystems/Thermofisher scientific Real-Time PCR System, use FAM, VIC/HEX/SUN, and ROX channels.

2.5. Result Interpretation

- Results are reported as MTBC Detected or MTBC Not Detected based on Ct/Cq values. Interpret the values for unknown samples based on the observations described in the following table. For invalid samples, there should be no amplification in the FAM and SUN/VIC/JOE channel for Internal control. **The Ct/Cq values of ≤38 Ct** for MTBC unknown samples should be considered as positive for the detection of MTBC.

FAM (MTBC)	SUN/VIC/JOE (IC)	Interpretation
+	+	MTBC detected
+	-	MTBC detected
-	+	MTBC not detected
-	-	Invalid

3. Limitations

- This assay is qualitative and does not differentiate MTBC species or detect non-tuberculous mycobacteria.

4. Quality Control

- Internal control included. External controls should be used as per lab policy.

5. Warnings, precautions and procedure limitations

- Protect the assays from light and store as indicated until ready for use. Excessive exposure to light can negatively affect the fluorescent probes of the assays.
- Use separate working places for sample preparation / nucleic acid extraction and amplification reactions.
- Never introduce an amplified product in reagent and/or nucleic acid extraction (sample preparation) area.
- Dispose of unused reagents and waste in accordance with laboratory guidelines.
- Use all necessary protective equipment (protective disposable gloves, a laboratory coat and eye protection) when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of the reagents when removing aliquots from reagent vials.
- Use RNase- and DNase-free filter pipette tips only. Use new tips for each pipetting step.
- Close the kit components' vials immediately after use and never interchange lids.
- Do not use reagents from damaged or leaking vials.
- The manufacturer is not responsible for the kit defectiveness due to incorrect handling.
- Read the whole Instructions for Use properly before setting up the assay starting the manipulation.
- Not following these instructions can lead to an erroneous result which can cause misdiagnosis or inappropriate treatment.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test
- Do not use the kit after its expiry date.
- The presence of UNG decontamination step reduces the risk of lower levels of carry-over amplicon contamination. However, contamination from very high levels of amplicon or by positive controls and/or clinical specimens can be avoided only by good laboratory practices and careful adherence to the procedures specified in these Instructions for Use.
- All reagents should be closely monitored for impurity and contamination.
- Discard the kit components empty vials, packaging material into the black color plastic bag and follow regulations for discarding.

6. Ordering information

- Catalogue number **– TBDx-25**
- **For ordering details visit** www.empediagnotics.com

7. Symbol Keys (referred from ISO 15223-1 standard guidelines)

Symbol	Description
	Product Catalogue Number
	Product Lot/ Batch Number
	Use by date
	Storage conditions
	Manufacturer of the product
	Pack size
	Consult instructions for use
	Research Use only
	Keep Dry
	Do not use if Package is damaged
	Keep away from Sunlight

- For more information on the symbols identified on the vials and packaging box, please refer ISO 15223-1 standard.

8. Regulatory Disclaimer

- For life science research only. Not for use in diagnostic procedures.

9. Support

- For help services please contact info@empediagnostics.com

EMPE DIAGNOSTICS

Corporate Headquarters
Stockholm, Sweden

Global Operations Center
Hyderabad, India