

Instructions for Use

Version: 1.0.1
Revision date: 17-Feb-26



Treponema pallidum (Syphilis) RT-PCR Kit

Catalog No.: abx472068

Size: 48 / 96 tests

Limit of Detection: 500 copies/ml

Storage: Store at 0°C to 35°C in the dark.

Application: For the qualitative detection of *Treponema pallidum* RNA in Human skin lesion and lymphocentesis fluid.

Introduction

Abbexa's *Treponema pallidum* RT-PCR Kit is a qualitative real-time fluorescent RT-PCR assay for the detection of *Treponema pallidum* RNA in Human skin lesion and lymphocentesis fluid. To achieve qualitative detection of *Treponema pallidum* at the nucleic acid level, the fluorescent quantitative PCR device has the ability to automatically generate a real-time amplification curve based on the detected fluorescent signal. A pair of primers and probes are included in the PCR mastermix for an internal amplification control. For each test run, the kit's positive and negative controls can be utilized as an external control. It is possible to monitor proper specimen collection, handling, and real-time PCR process using the outcomes of the internal and external control.

Kit Components:

48 tests

1. Syphilis Mix: 48 vials
2. Positive Control: 1 vial
3. Negative Control: 400 µl
4. PCR strip caps: 6 × 8
5. Hermetic bag: 1

96 tests

1. Syphilis Mix: 96 vials
2. Positive Control: 1 vial
3. Negative Control: 400 µl
4. PCR strip caps: 12 × 8
5. Hermetic bag: 1

Materials Required But Not Provided

1. Biosafety cabinet
2. Viral Nucleic Acid Extraction System and Extraction Kit
3. Real time PCR system
4. Vortex mixer
5. Sample preservation solution
6. Pulse centrifuge with strip tube attachment
7. Real time PCR reaction tubes (0.2 ml)
8. Ice container
9. Transfer pipettes (0.5 µl – 1000 µl)
10. Sterile filter tips for transfer pipettes
11. Sterile tubes
12. Sterile swabs
13. Biohazard waste container
14. Cold storage (refrigerator and freezer)

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Protocol

A. Sample Collection and Preparation

1. Specimen Collection

- **Skin lesion fluid:** Gently swab the lesion site 10 – 20 times, rotating the swab as you do so. Immediately place swab into a storage tube containing sample preservation solution, making sure the submerge the swab head. If the swab sticks out of the tube, carefully break the handle and discard. Close the lid of the storage tube and shake 10 – 20 times to ensure thorough immersion of the swab head in the sample preservation solution.
- **Lymphocentesis fluid:** Using a 1 ml sterile syringe, aspirate sterile isotonic saline, approximately 0.25 ml – 0.5 ml and the puncture the lymph node. Inject the saline before inhaling the saline back into the syringe. Repeat the injection and inhalation 2 – 3 times. On the final inhalation, remove the fluid and inject the fluid into the sample preservation solution.

2. Specimen Storage

It is recommended that samples are tested immediately. Untested samples may be stored at 2–8°C for up to 24 hours, stored at -20°C for up to 10 days, or at -70°C for longer term storage.

It is important to store specimens properly to avoid damage to the sample. Repeated thawing and refreezing can lead to erroneous results.

Note:

- Keep separate areas for preparing specimens, storing and preparing chemicals, and amplifying data to avoid cross-contaminating specimens.

3. Specimen Preparation

Extract the nucleic acid from at least 300 µl of specimen using the preferred Viral Nucleic Acid Extraction System and Extraction Kit. 60 – 100 µl of extracted RNA solution is recommended for detection. The extracted RNA solution should be tested immediately or stored at -20°C.

B. Assay Procedure

For each of the sections below, perform the protocol in a different area in the lab. This reduces the chance of cross-contamination between samples and reagents. This assay is extremely sensitive to cross-contamination.

1. Reaction Preparation

- **Syphilis Mix:** Each reaction tube contains lyophilized Syphilis Mix. The samples, positive and negative controls will be added to these tubes. Mark these tubes for each sample, positive, and negative control. Each PCR run requires a positive and negative control.
- **Negative Control:** The Negative Control can be used directly.
- **Positive Control:** To reconstitute, add 200 µl of Negative Control to the Positive Control and mix thoroughly using a vortex mixer for 20 – 30 seconds. Store prepared control at -20 °C.

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- Once opened, if not used, the Syphilis Mix reaction tubes and silica can be placed into the hermetic bag and well-sealed. Store at 2- 8°C for up to 2 weeks
- All other components must be stored at -25 – -15°C in the dark, after first use.
- PCR Reagents should be fully thawed prior to use. Avoid freeze/thaw cycles.

2. Addition of Samples

- To each of the pre-marked PCR tubes, add 19 µl of processed sample, Positive Control, or Negative Control.
- Cap the PCR reaction tubes using the PCR strip caps and ensure the tubes are completely sealed.
- Mix thoroughly using a vortex mixer, then centrifuge at 2000 rpm – 6000 rpm briefly to bring down all the liquid.

Note:

- Ensure the reaction tube solution is completely clarified, with no bubbles before testing. If precipitate or bubbles remain in the reaction tubes, vortex and centrifuge again.

3. Amplification

- Place the PCR reaction tubes (reaction volume: 25 µl) into the PCR instrument, configure the settings, and initiate the specified cycling protocol.

Step	Cycles	Temperature (°C)	Time (s)	Note
1	1	50	900	Reverse Transcription
2	1	95	60	Initial Denaturation
3	40	95	5	Denaturation
4		60	25	Annealing and Extension

Note:

- For ABI Real – Time PCR systems, step 4 should be altered to 30 seconds.

2. Settings of fluorescence detection:

Channel	Target Gene
FAM	<i>Treponema pallidum</i> RNA
VIC	Internal Control

- Adjust the internal reference parameter for fluorescence on the instrument to "None." For instance, in the case of the ABI Real-Time PCR system, set the "Passive Reference" setting to "None."
- Initiate the PCR cycler in accordance with the instructions provided in its user manual.

C. Result Analysis

Establish the start value baseline within the range of 3-15 and the end value baseline within the range of 5-20, allowing for adjustments based on the specific circumstances. The principle for setting the fluorescence threshold is to position the threshold line just above the peak value of the negative control amplification curve. The negative control is expected to show no amplification, late cycle threshold (Ct) values are considered background noise. Analyze and interpret the results.

Validation of test

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First test the positive and negative controls with the FAM/ VIC channels to determine if the test is valid:

Positive and Negative Controls	Syphilis gene (FAM)	Internal Control (VIC)	Results
Positive Control	Ct ≤ 40 with identifiable S-type amplification curve	Ct ≤ 36 with identifiable S-type amplification curve	Valid
Negative Control	Ct > 40 or no identifiable S-type amplification curve	Ct > 36 or no identifiable S-type amplification curve	Valid

Interpretation of test results

After testing that the controls are valid, the samples can be tested with the FAM/ VIC channels. The test results are evaluated based on the table provided below.

Syphilis Gene (FAM)	Internal Control VIC	Results
Ct ≤ 40 With an identifiable S-type amplification curve	Result presence/ absence does not affect positivity determination.	Syphilis Positive
CT > 40, With no identifiable S-type amplification curve	CT ≤ 36, With identifiable S-type amplification curve	Syphilis Negative
CT > 40, With no identifiable S-type amplification curve	Ct > 36 or no identifiable S-type amplification curve	Invalid

Precautions:

1. This kit is for research use only and the results are for reference only. It is recommended to use this kit in conjunction with another detection method.
2. Negative results do not preclude *Treponema pallidum* infection.
3. This kit is for qualitative detection of *Treponema pallidum* in Human skin lesion and lymphocentesis fluid. For other sample types, a preliminary experiment is recommended to determine compatibility with this kit.
4. Viral Nucleic Acid Extraction kits require testing on samples beforehand to verify their suitability.
5. All waste should be disposed of appropriately. Please note that you may need to follow special waste disposal procedures for infectious samples. Please check local disposal regulations.
6. Samples should be labelled and shipped in accordance to testing laboratory requirements.
7. Keep separate areas for preparing specimens, storing and preparing chemicals, and amplifying data to avoid cross-contaminating specimens.

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.