A Real-Time PCR (qPCR) Assay for Detection of Leptospira spp

Product Insert

*ab*TES™ Leptospira qPCR I Kit
Kit Version: 1.0

RUO For research use only

REF 300301 (50 Reactions)
300302(100 Reactions)

Store at -25°C to -15°C

AITbiotech Pte Ltd
25 Pandan Cresent, #05-15,
TIC Tech Centre,
Singapore 128477

AITbiotech
Advanced • Innovative • Trusted

For use on Bio-Rad-CFX96, QIAGEN RG Q and ABI 7500.

1. Pathogen Information

Leptospirosis is a widespread bacterial zoonotic disease in humans caused by pathogenic species of the genus *Leptospira*. The clinical spectrum of the infection ranges from sub clinical to severe illness with high mortality rate. Human Leptospirosis causes severe multi organ dysfunction that may end in multi organ failure and death. More than 200 *Leptospira interrogans* and other *Leptospira* species serovars have been identified as pathogenic. The pathogenic and non-pathogenic *Leptospira* species are indistinguishable morphologically or by growth characteristics. It has long been known that Leptospires spreads almost immediately from the site of entry of skin or mucous membrane, via lymphatics to the blood stream, where they circulate to all tissues. Early diagnosis is essential as the antibiotic treatment is most effective when it is initiated early in the course of disease. Failure to recognize acute leptospirosis means a delay in the initiation of its therapy and possibly ensuing severe complications such as Weil's syndrome. If the host survives the acute infection, septicemia and multiplication of organism persist until the development of immunoglobulin in plasma, followed by rapid immune clearance.

2. Test Description

The *ab*TES™ Leptospira qPCR I Kit is a real-time polymerase chain reaction (qPCR) kit for the detection of the pathogenic *Leptospira* species. The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of the all pathogenic *Leptospira* species using highly specific primer pairs and double-dye hydrolysis probes. The recommended sample types are EDTA blood (heparin blood is not recommended) and serum. An **Internal Control (IC)** is also supplied to check for possible PCR inhibition.

3. Storage Conditions

The components of *ab*TES™ Leptospira qPCR I Kit should be stored in the dark, at -20°C in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents are used intermittently, it is suggested to keep the reagents frozen in aliquots.

4. Kit Components

Tubes	Items	300301 (50 rxns)	300302 (100 rxns)
1	Primer/Probe Mix	1x 50 µL	2x 50 µL
2	Enzyme/Reaction Mix	1x 500 µL	2x 500µL
3	Internal Control	1x 200 µL	2 x200 µL
4	Leptospira Positive control	1x 100 µL	1x 100 µL
5	Nuclease-free Water	1x 600 µL	2x 600 µL

5. Additional Materials Required but not Provided

- Disposable powder-free gloves
- Nucleic acid extraction Kit
- Vortex mixer
- Pipettes and pipette tips with filter
- Desktop centrifuge with rotor
- Real-time thermal cycler
- 0.2 mL PCR tubes/ 96-well PCR plates
- 0.1mL strip tube & cap (for QIAGEN RG Q only)
- Ice box/cooling block

6. Limitations and General Precautions

- The use of this product and its data interpretation are intended for personnel trained in real-time PCR techniques and *in vitro* diagnostics procedures only.
- It is advisable to analyze the real time PCR graph at the end of the run to determine the validity of the Ct data.
- Appropriate specimen collection, transport, storage and nucleic acid extraction procedures are required for reliable results.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Store and extract positive materials (specimens, controls and amplicons) separately from all other reagents and add to the reaction mix in a separate facility, if possible.
- Thaw all the components thoroughly at room temperature before starting an assay.
- When the components are thawed, mix the components and centrifuge briefly.
- Do not use the kit after its expiration date.

7. Procedures

7.1. Nucleic Acids (NA) Extraction

Standard NA extraction kits are compatible with this assay. Please carry out NA extraction as per instructed in the manufacturer's Extraction Kit manual.

7.2. PCR Reaction Setup

Thoroughly thaw all components, mix and spin briefly. Keep all components and samples on ice.

Prepare the PCR reaction based on the following pipetting scheme:

Reagent	Volume per reaction			
	Test sample reaction	Positive control reaction	Negative control reaction	Non template reaction
Enzyme/Reaction Mix	10 µL	10 µL	10 µL	10 µL
Primer/Probe Mix	1 µL	1 µL	1 µL	1 µL
Nuclease-Free Water	3 µL	3 µL	8 µL	9 µL
Internal Control	1 µL	1 µL	1 µL	-
Positive Control	-	5 µL	-	-
Extracted Test Sample	5 µL	-	-	-
Total Volume	**20 µL**	**20 µL**	**20 µL**	**20 µL**

7.3. PCR Cycling Conditions

The following cycling conditions were established and validated on Bio-Rad CFX96, QIAGEN RG Q and ABI 7500. You may need to adjust these conditions for other real-time platforms. **Green/FAM** (Leptospira) and **Orange/Texas Red** (IC) channels should be chosen and the fluorescence is measured at the end of annealing-extension phase of each cycle.

Phase	Description	No. of Cycles	Temperature	Duration
1	*Taq* activation	1	95 °C	2 min
2	Amplification	45	95 °C	10 sec
			*53 °C	30 sec

**Data acquisition at annealing phase*

8. Interpretation of Data

A sample is considered as positive if the fluorescence level is higher than the threshold value **and** has a Ct value of ≤44. Samples with a Ct value of >44 should be regarded as negative.

As the kit contains an internal control, all specimens that are negative for Leptospira (Green/FAM) should be positive at the internal control channel (Orange/Texas Red). A negative internal control in this case may indicate a presence of PCR inhibitors in the sample, a problem during the extraction step or a problem with the PCR reaction. The internal control may not necessarily be positive if the sample is positive for Leptospira due to competition of reagents.

Result	Leptospira (Green/FAM)	Internal Control (Orange/Texas Red)
Negative	>44 or -	+
Positive	≤44	+ or -
Indeterminate	>44 or -	-

9. Troubleshooting

9.1. No signal observed with positive control

- Check programmed temperature settings against the protocol given.
- Affirm if proper storage was done and check the expiry date on the kit; Repeat the run with a new kit if needed.
- PCR inhibition has possibly occurred: re-purify DNA sample to remove inhibitors and repeat PCR, if needed.

9.2. Signal detected for negative control

- A contamination in the reagents or samples is highly possible.
- Repeat experiment protocol and take steps to locate source of contamination.

9.3. Weak or no signal of the internal control and no sign detection in analytical channel as well

- A possible PCR inhibition has occurred. Re-extract the sample to remove inhibitors and repeat PCR, if needed.
- Affirm if proper storage was done and check the expiry date on the kit. Repeat the run with a new kit if needed.

10. Explanation of Symbols

RUO	For research use only
REF	Catalogue number
	Store at -25°C to -15°C
	Manufacturer
LOT	Lot number
	Use by
	Contains sufficient for <n> tests

Electronic copy of the product insert can be downloaded at http://aitbiotech.com/leptospira/

AITbiotech Pte Ltd
25 Pandan Crescent #05-15, TIC TECH Centre, Singapore 128477
(65) 6778 6822 • sales@aitbiotech.com • www.aitbiotech.com