AstraArt Leptospirosis qPCR Kit

PRINCIPLE

AstraArt Leptospirosis qPCR Kit is a one-step real-time polymerase chain reaction assay for the qualitative detection of *Leptospira spp*. DNA in biological samples. The assay is a combination of the latest advanced buffer chemistry, PCR enhancers and stabilizers along with hot-start Taq polymerase, dNTPs and MgCl2. This assay has been designed for highly reproducible, accurate results in the presence of inhibitors, making it ideal for detection of lowest copy numbers of DNA.

Nucleic acid obtained from samples collected will be amplified with primer probe designed towards Chromosomal gene Target and EIC synthetic control gene.

PACKAGE CONTENTS Specification Quantity 100 tests Description qPCR Master Mix qPCR amplification Mix $1000\mu\ell \ge 1$ tube Primer Mix Target specific Primer Probe mix $1000\mu\ell \ge 1$ tube Positive Control (PC) Positive Control 500µl x 1tube Negative control (NC) No Template Control 1000µl x 1 tube Exogenous Internal Control (EIC) Extraction control 1000µl x 1 tube Catalogue Number AG/PLEP/23/01

STORAGE & STABILITY

- All the reagents should be stored at -20 °C. Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. Avoid repeated freeze/thaw cycles for reagents.

SAMPLE REQUIREMENTS

- Specimens collected for Leptospirosis DNA investigation should be refrigerated (2 to 8°C) or frozen (-20°C or lower) within one hour after collection.
- If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20°C or lower.
- Longer term specimen storage (>60 days from collection) is recommended at -70°C
- It is ideal to carry out extraction protocol with fresh samples.
- Repeated freezing and thawing should be avoided.
- Transport the specimens at a temperature between 2 to 8°C.

SAMPLE COLLECTION

- Blood samples can be collected.
- Follow universal precautions. All specimens should be considered as potentially infectious and handled accordingly.
- Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when handling specimens and kit reagents.

ASSAY PROCEDURE:

Nucleic acid along with EIC ($10\mu\ell$ per sample) is isolated from the sample using Nucleic acid extraction system and is amplified using the AstraArt Leptospirosis qPCR Kit on the Real-time PCR Instrument system. In the process, the probe anneals a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the Real-time PCR Instrument system.

a. Preparation of Real-time PCR reagents: Briefly Centrifuge all the reagents. Prepare the reagents according to the table below. The final volume is calculated by multiplying the number of samples by the volume of each component in Table 1 accordingly.

Table 1: Components of Master mix:

Components	
qPCR Master Mix	$10\mu\ell$
Primer Mix	$10\mu\ell$
Total Volume	20µl

b. Mix the reaction master mix and spin-down briefly. Aliquot $20\mu\ell$ of the master mix into each well of 96-well plate and add $10\mu\ell$ of the sample DNA/Negative control/Positive control/Negative extraction control accordingly. Seal the plate and spin-down briefly. Run the Protocol immediately on the Real-time PCR instrument with following cycling conditions in Table 2.

Table 2: Cycling Conditions:

Steps	Temperature °C	Time	Cycle			
1	95	2 minutes	1			
2	95	5 seconds				
3	63	10 seconds	5			
4	67	10 seconds				
5	95	5 seconds				
6	63*	10 seconds	40			
7	67	10 seconds				
	Leptospira spp. DNA uses VIC/HEX yellow channel; Exogenous Internal Control uses FAM Green ch					
	*Fluorescence measured at 63° C.					



Note: Please select "None" in both Passive reference and Quencher.

c. Interpretation of Results:

Interpret the values for unknown samples based on the observations as described in the following table. \leq 38 Ct of unknown samples should be considered for result interpretation. The lower limit of detection (LOD) of AstraArt Leptospirosis qPCR Kit is defined as 5 copies/reaction.

Table 3: Determination of CT value:

Fluorescent Channel	CT value	Result
EIC (FAM)	≤ 38	+
Leptospira spp. DNA in HEX/VIC	≤ 38	+

Table 4: Conclusion:

results	EIC (FAM)	Leptospira spp. DNA (HEX/VIC)	Conclusion
Negative Extraction Control	+	-	Valid
Negative Control	-	-	Valid
Positive control	+	+	Valid
Sample	+	+	Positive
Sample	-	+	Positive
Sample	+	-	Negative
Sample	-	-	Invalid run repeat test with fresh sample

LIMITATION OF THE PROCEDURE:

- 1. AstraArt Leptospirosis qPCR Kit is used for qualitative detection of Leptospirosis DNA from specimens.
- 2. The specimen to be tested shall be collected, processed, stored, and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
- 3. Extraction and amplification of nucleic acid from samples must be performed according to the specified methods listed in this procedure.
- 4. The kit has been validated for the following Real-Time PCR instruments: CFX96TM Deep Well Real-Time PCR Detection System (Bio-Rad), CFX96TM Deep Well Dx System (Bio-Rad), CFX96TM Real-Time PCR Detection System (BioRad), CFX96TM Dx System (Bio-Rad), CFX96TM Real-Time PCR Detection System (Bio-Rad), CFX96TM Data System (Bio-Rad), CFX96TM Real-Time PCR System (Applied Biosystems).

WARNING & PRECAUTIONS:

- Do not use the product if there is evidence of leakage.
- Adhere to standard procedures and published protocols for sample collection, processing, and disposal.

SYMBOLS:



CE mark of Conformity



Refer to the instructions 13485:2016 ISO

ISO





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